FORMULATION AND EVALUATION OF A MEDICATED NAIL LACQUER FOR THE TREATMENT OF ONYCHOMYCOSIS

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THIS IS TO CERTIFY THAT Ms.ASWANI.V.M HAS DONE HER M.PHARM SECOND YEAR DISSERTATION TITLED, "FORMULATION AND EVALUATION OF A MEDICATED NAIL LACQUER FOR TREATMENT OF ONYCHOMYCOSIS". SHE WAS FOUND TO BE HARD WORKING AND SINCERE DURING THIS PERIOD. WE WISH HER THE VERY BEST FOR ALL FUTURE ENDEAVOURS.

For hikma herbs

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Abstract

ABSTRACT

Onychomycosis (also known as dermatophytic onychomycosis or *Tinea unguium* is a fungal infection of the nail. The causative pathogens of onychomycosis include dermatophytes, *Candida*, and nondermatophytic molds. In the present work, a medicated antifungal nail lacquer of Miconazole nitrate has been developed. The objective of the study was to deliver a sustained release of Miconazole nitrate over extended period of time up to 48 hours, and hence reduce the frequency of administration. This was expected to improve clinical efficacy and also improve the patient compliance. The nail lacquer formulation were prepared by simple mixing and analyzed for non -volatile content, gloss, smoothness to flow, drug diffusion studies, drug content estimation, anti -microbial studies. Among all formulation, nail lacquer prepared with 2% Miconazole nitrate, 6% nitrocellulose, 1% ethyl cellulose, 15 % salicylic acid, 10% propylene glycol and 10% 2-H- β-CD exhibited good non- volatile content, drug release, drug content estimation and zone of inhibition. The drug release could be extended up to 48 hour and a complete release of 98.12% was observed. FTIR studies reveled that drug and excipients are compatible. Accelerated stability study of selected optimized formulation, F11 was done as per ICH guidelines for 1 month at 40±20C, which revealed that no significant change with respect to the initial characteristics was observed. Formulation and usage of these systems are considered to be safe, without any complication. So we can conclude that the antifungal nail lacquer may be one of the novel dosage forms that can revolutionize the pharmaceutical and health care sector.

LIST OF ABBREVIATIONS

mm	Millimeter
µ	Micron
%	Percentage
e.g.	Example
OM	Onychomycosis
PAS	Periodic acid Schiff
MIC	Minimal inhibitory concentration
FDA	Food and drug administration
h or hr.	Hour
pH	Hydrogen ion concentration
Da	Dalton
SH	Sulfhydryl group
SC	Subcutaneous
V/cm	Volt per centimeter
HP- β- CD	Hydroxyl propyl- β-cyclodextrin
λmax	Wave length of maximum absorption
FTIR	Fourier Transform Infrared
CAS No	Chemical abstracts service
°C	Degree centigrade
g/mol	Gram per mol
ICH	International Conference on Harmonisation
mg	Milligram

mPa S	Millipascal second
PO	Per os (by mouth)
ml	Milliliter
CP	Centipoise
USP	United states pharmacopoeia
°F	Degree farenheat
mmHg	Millimeters of mercury
PG	Propylene glycol
AR grade	Analytical reagent grade
BP	British Pharmacopoeia
JP	Journal of Perinatology
USPNF	United states pharmacopoeia and National Formulary
OTC	Over the Counter
uv	Ultraviolet
M	Molar concentration
PBS	Phosphate buffer solution

RH	Relative humidity
MALDI-TOFMS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
IP	Indian Pharmacopoeia
IR	Infrared
Sec	Seconds

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Introduction

Aim and objective

Review of Literature

Drug and Polymer Profile

Materials and Methods

Results and Discussion

Summary

Conclusion

Bibliography

1.INTRODUCTION

Over the last decades the treatment of illness has been accomplished by administrating drugs to human body via various routes namely oral, parental, topical, inhalation etc. Every medical condition demands an accurate and appropriate treatment. As a matter of fact, the thought of resolving the patient's disease with least harm done to the patient's health is said to be the basic goal of any therapy. Moreover a good treatment technique necessitates thorough knowledge of pharmacokinetics and pharmacodynamics of the intended drug. Hence we struggle day to day relentlessly to research and better our techniques and technology to develop with the best mode of treatment ensuring fast recovery as well as assuring safety of the patient.

Human nails do not have only protective and decorative role, but can also be considered as an alternative pathway for drug delivery, especially in nail diseases such as onychomycosis or psoriasis. These nail diseases are widely spread in the population, particularly among elderly and immune-compromised patients. Although the architecture and composition of the nail plate severely limits penetration of drugs and in addition to that only a fraction of topical drug penetrates across the nail, oral therapies are accompanied by systemic side effects and drug interactions. For the successful treatment of nail disease the applied active drug must permeate through the dense keratinized nail plate and reach deeper layers, the nail bed and the nail matrix [1]. The inadequate research and knowledge regarding the properties of keratinized nail plate, the nail bed and the nail matrix caused a lesser focus on ungual system.

Horny structure nail plate is responsible for penetration of drug across it. As it is hard enough the penetration becomes difficult, only a fraction of topical drug penetrates across it. Hence the effective therapeutic concentration is not achieved. The nail plate may appear abnormal as a result of decreased glow. It is due to the involvement of nail bed, reduction of blood supply, physical or chemical features of nail bed. As a result variety of diseases occurs. These diseases can be cured by achieving desired therapeutic concentration of drug by nail drug delivery system.[2]

Major challenges of drug delivery to the nail (ungual drug delivery), with the lack of understanding of both the barrier properties of the nail and formulations to achieve enhanced ungual delivery restricting the efficiency of topical treatments for nail disorders. And also suffer from low patient compliance due to the long treatment periods (up to 4-8 months) which are required.

However, existing oral formulations typically contain large doses of active ingredients and also require long treatment, creating the potential for systemic toxicity especially in the liver. Thus, developing more effective methods for nail drug delivery is an important objective for the pharmaceutical industry.[3]

1.1 STRUCTURE OF THE HUMAN NAIL[4]

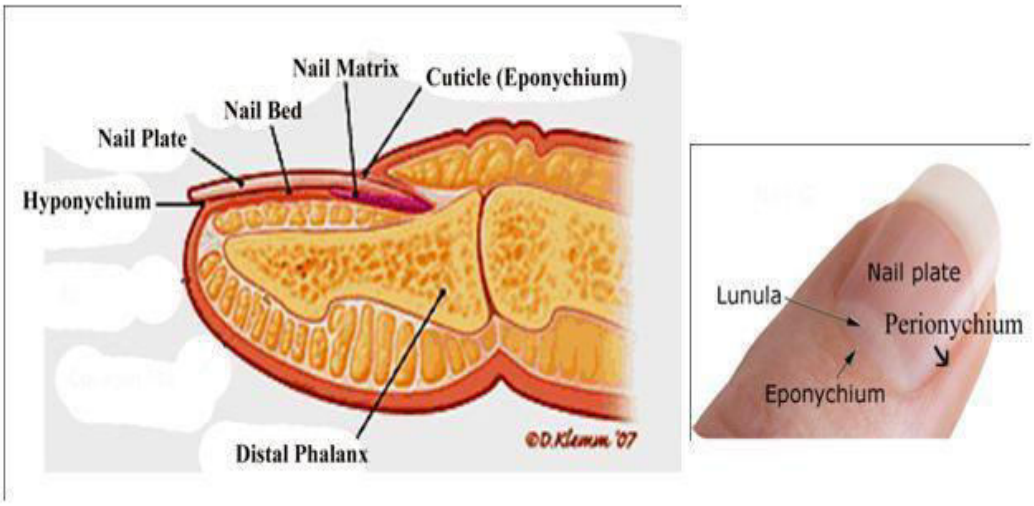

Figure 1: Structure of human nail

The human nail consists of

- Nail matrix or the root of the nail
- Nail bed
- Eponychium or cuticle
- Paronychium
- Hyponychium
- Nail plate
- Lunula [5]

a) Nail Root.

The root of the fingernail is also known as the germinal matrix. This portion of the nail is actually beneath the skin behind the fingernail and extends several millimeters into the finger. The fingernail root produces most of the volume of the nail and the nail bed. This portion of the nail does not have any melanocytes, or melanin producing cells. The edge of the germinal matrix is seen as a white, crescent shaped structure called the LUNULA.

b) Nail Bed.

The nail bed is part of the nail matrix called the sterile matrix. It extends from the edge of the germinal matrix (lunula) to the hyponychium. It is a thin, soft, noncornified epithelium, connected with the ventral layer of the nail plate and underlying papillary dermis and contains the blood vessels, nerves, and melanocytes, or melanin-producing cells. As the nail is produced by the root, it streams down along the nail bed, which adds material to the undersurface of the nail making it thicker.

c) Cuticle / Eponychium

The cuticle of the fingernail is also called the eponychium. The cuticle is situated between the skin of the finger and the nail plate fusing these structures together and providing a waterproof barrier.

d) Perionychium

The perionychium is the skin that overlies the nail plate on its sides. It is also known as the paronychial edge. The perionychium is the site of hangnails, ingrown nails, and an infection of the skin called paronychia.

e) Hyponychium

The hyponychium is the area between the nail plate and the fingertip. It is the junction between the free edge of the nail and the skin of the fingertip, also providing a waterproof barrier.

f) Lunula

The edge of the germinal matrix is seen as a white, crescent shaped structure called the LUNULA.

Nail Growth

Nails grow all the time, but their rate of growth slows down with age and poor circulation. Fingernails grow faster than toenails at a rate of 3mm per month. It takes 6 months for a nail to grow from the root to the free edge. Toenails grow about 1 mm per month and take 12-18 months to be completely replaced.

Thickness of finger nail is 0.25 - 0.6 mm and that of toe nail is up to 1.3mm. The nail plate is made up of approximately 25 layers of dead keratinized, flattened cells. They are strongly bound to one another via numerous intercellular links, membrane-coating granules and desmosomes. Desmosomes are cell structures, specialized for cell-to-cell adhesion and randomly arranged on the lateral sides of plasma membranes. The fingernail has a three-layer structure (from outer to inner) –

- **dorsal**
- **intermediate and**
- **Ventral layers** with a thickness ratio of approximately 3:5:2,respectively.

The dorsal outer layer is dense and hard, consisting of cornified keratin only a few cells thick (approximately 200 μ). *The intermediate layer,* in contrast to the dorsal layer, shows highly fibrous structure oriented in a direction perpendicular to the direction of nail growth and constitutes roughly 75% of the plate's thickness. *The ventral layer* is very thin and consists of a few layers of cells which connect the nail plate to the nail bed below.

1.2 ANATOMY OF HUMAN NAIL[6]

The human nail plate consists of three layers; the dorsal and intermediate layer derived from the matrix and the ventral layer from nail bed. The intermediate layer is three - quarter of the whole nail thickness & consists of the soft keratin. The upper layer, dorsal, is only a few cell layer thick but consists of hard keratin, with relatively

high sulphur content, mainly in the form of amino acids cysteine, which constitutes 94% by weight of nail. The upper layer of the nail mainly diffuses into and through the nail plate. The ventral layer consists of soft hyponychial in which many pathological changes occur. Thus, in the treatment of these nail diseases, an effective drug concentration in the ventral nail plate would be of great importance.

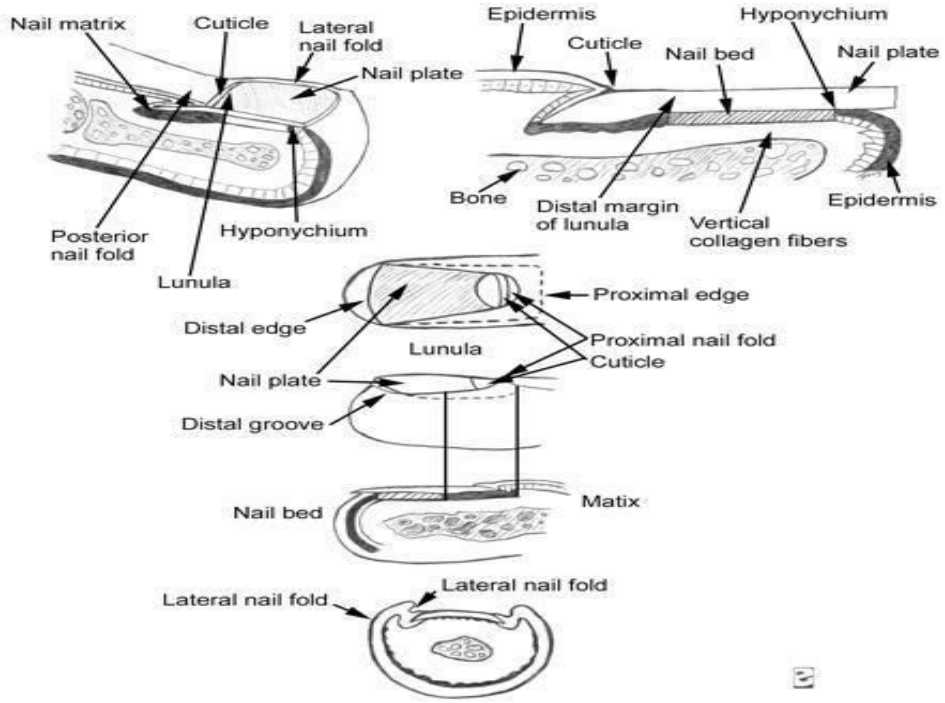

Figure 2: Nail Anatomy[6]

Nail growth is greatest in childhood and decrease slowly with aging. Due to pressure from posterior nail fold grows forward instead of upward. Nail growth is also affected by local disturbances in the nail fold or by abnormal keratinization of the nail plate. General or local factor may result in the development in the nail of thicken, ridging, pitting, discoloration, brittleness, splitting and even separation of nail from its bed (onycholysis). A transverse groove may result from severe illness. The changes in colour for a variety of reasons for instance white spots in the nail plate, which is seen 62% of normal people, is due to imperfect keratinization with retention of nuclear material.

1.3 CHEMICAL PROPERTIES OF THE NAIL PLATE[7]

- Low sulfur keratins embedded in an amorphous matrix of high -sulfur proteins rich in cysteine.

- Water content is 20%
  - If <18% = brittle
  - If >30% = opaque and soft
  - Lipid content is <5% mainly cholesterol
- Trace inorganic elements:
  a) Iron
  b) Zinc
  c) Calcium
- Nail keratins:
  a) 80% of keratins are hard "hair-type" keratins
  b) 20% of keratins are soft "skin-type" keratins

1.4 NAIL DISEASES

The nail plate may appear abnormal as a result of, a congenital defect, disease of skin with involvement of the nail bed, systematic disease, reduction of blood supply, local trauma, tumors of the nail fold or nail bed, infection of the nail fold, infection of the nail plate.

There are many nail diseases such as

A) **Green-nail syndrome** : An infection which is caused by Pseudomonas [7]

B) **Paronychia**:

1. **Acute paronychia**: Erythema, swelling and throbbing pain in the nail fold caused by bacterial infection, **e.g.** S. aureus and Group A streptococci.
2. **Chronic paronychia**: Mostly occurs in patients whose hands are constantly in water with repeated minor trauma damaging the cuticle so that irritants can further damage the nail fold. Commonly becomes infected especially with C. albicans or Pseudomonas spp. (produces a green or black discoloration)[8]

C) **Parakeratosis pustulosa:** Showing subungual hyperkeratosis and onycholysis [9, 10]

D) **Nail Psoriasis**: Scaly skin, the nail plate becomes pitted, dry and often crumbles and also appears red, orange or brown, with red spots in the lunula.

E) **Yellow nail syndrome**: A rare condition characterized by yellow nails with lack of cuticle, grows slowly, and is loose or detached associated with onycholysis in one or more nails[11].

F) **Onychomycosis [2, 3]:** Onychomycosis accounts for one third of integumentary fungal infections and one half of all nail disease. Tinea unguium is more than a cosmetic problem, although persons with this infection are often embarrassed about their nail disfigurement. Because it can sometimes limit mobility, onychomycosis may indirectly decrease peripheral circulation, thereby worsening conditions such as venous stasis and diabetic foot ulcers. Fungal infections of the nails can also spread to other areas of the body and, perhaps, to other persons[12,14].

1.5 ETIOLOGY[13]

The causative pathogens of onychomycosis include dermatophytes, Candida, and nondermatophytic molds. Dermatophytes are the fungi most commonly responsible for onychomycosis in the temperate western countries, while Candida and nondermatophytic molds are more frequently involved in the tropics and subtropics with hot and humid climate.

Dermatophytes

Trichophyton rubrum is the most common dermatophyte involved in onychomycosis. Other dermatophytes that may be involved are Trichophyton interdigitale, Epidermophyton floccossum, Trichophyton violaceum, Mycosporum gypseum, Trichophyton tonsurans and Trichophyton soudanense.

Other pathogens

Other causative pathogens include Candida and non dermatophytic molds, in particular members of the mold generation Scytalidium (name recently changed to Neoscytalidium), Scopulariopsis. Candida mainly causes fingernail onychomycosis in people whose hands are often submerged in water. Scytalidium mainly effects people in the tropics, though it persists if they later move to areas of temperate climate.

Risk factors:

Risk factors for Onychomycosis include family history, increasing age, poor health, prior trauma, warm climate, participation in fitness activities, immunosuppression (e.g., HIV, drug induced), communal bathing, and occlusive footwear.

1.6 CLASSIFICATION OF ONYCHOMYCOSIS [2, 3]

A. Distal Subungual Onychomycosis

The most common form of Tinea unguium is distal subungual. Distal subungual onychomycosis may develop in the toenails, fingernails or both. The infection is usually caused by Trichophyton rubrum, which invades the nail bed and the underside of the nail plate, beginning at the hyponychium and then migrating proximally through the underlying nail matrix. Susceptibility to distal superficial onychomycosis may occur in an autosomal dominant pattern within families (5A).

B. White Superficial Onychomycosis

White superficial onychomycosis accounts for only 10 percent of onychomycosis cases. White superficial onychomycosis is caused by certain fungi that directly invade the superficial layers of the nail plate and form well-delineated opaque "white islands" on the plate. As the disease progresses, these patches coalesce to involve the entire nail plate. The nail becomes rough, soft and crumbly. The most common causative agent is Trichophyton mentagrophytes. This type of onychomycosis can be treated with topical antifungal drugs alone. (5B)

C. Proximal Subungual Onychomycosis

Proximal subungual onychomycosis occurs when the infecting organism, usually *T. rubrum*, invades the nail unit through the proximal nail fold, penetrates the newly formed nail plate and then migrates distally. Fingernails and toenails are equally affected. This form of onychomycosis usually occurs in immune compromised persons and is considered a clinical marker of human immunodeficiency virus infection.

Proximal subungual onychomycosis can also arise secondary to local trauma (5C).

D. Candidal Onychomycosis

Candida onychomycosis can be divided into three general categories.

(i) Infection beginning as a paronychia (infection of the structures surrounding the nail; also called a "whitlow"), the most common type of Candida onychomycosis.

(ii) Patients with chronic mucocutaneous candidiasis are at risk for the second type of Candida onychomycosis, called Candida granuloma, which accounts for less than 1% of onychomycosis. This condition is seen in immune compromised patients and involves direct invasion of the nail plate.

(ii) Candida onycholysis can occur when the nail plate has separated from the nail bed. Distal subungual hyperkeratosis can be seen as a yellowish gray mass lifts off the nail plate.

E. Total Dystrophic Onychomycosis

Total dystrophic onychomycosis may be the end result of any of the four main forms of onychomycosis (5D).

Figure 3: Classification of onychomycosis

1.7 CLINICAL FEATURES[13]

The nail plate can have a thickened, yellow or cloudy appearance. The nails can become rough and crumbly, or can separate from the nail bed. There is usually no pain or other bodily symptoms, unless the disease is severe. Dermatophytids are fungus-free skin lesions that sometimes form as a result of a fungus infection in another part of the body.

This could take the form of a rash or itch in an area of the body that is not infected with the fungus. Dermatophytids can be thought of as an allergic reaction to the fungus. People with onychomycosis may experience significant psychosocial problems due to the appearance of the nail. This is particularly increased when fingernails are affected.

1.8 DIAGNOSIS OF ONYCHOMYCOSIS[14, 15, 16]

Conventional methods for identifying fungal organisms in the nail plate of patients with onychomycosis (OM) include direct microscopy (after potassium hydroxide solution incubation), fungal culture, and histopathology (using Periodic Acid Schiff [PAS] stain). Surgical pathology testing (of the subungual nail bed and/or the nail plate) using PAS stain is the current gold standard (approaching 100% sensitivity) for the diagnosis of OM. Newer methods for diagnosing OM include polymerase chain reaction (which has a very high specificity), optical coherence tomography, confocal laser scan microscopy, matrix- assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and phase contrast hard x-ray microscopy. Confirmation of observations and availability and cost must be considered before these newer methods for diagnosing OM can be incorporated in clinical practice.

1.9 TREATMENTS OF ONYCHOMYCOSIS[17, 18, 19]

Several modalities can be used for the treatment of onychomycosis topical therapy, systemic therapy, combination therapy, and nail removal. Patients greater than 55 years of age may have a higher rate of relapse.

1.9.1 Nail removal, avulsion

Removal of diseased nails can be used as an adjunctive therapy but not as the sole therapy for onychomycosis. Surgical nail avulsion is rarely used to treat onychomycosis in diabetic patients because of their increased risk for secondary infections, gangrene, and poor wound healing. However, in severe or refractory cases, nail removal may be used. It may also be used when oral therapy is contraindicated or ineffective.

Demerits of surgical treatment

Surgical nail avulsion can bring severe pain and further trauma to the patient. Surgical removal of the nail plate (fingernail or toenail) is not effective treatment of onychomycosis without additional therapy. This procedure should be considered only an adjunctive treatment combined with oral medical therapy.

1.9.2 Oral therapy

Many studies have evaluated systemic treatments for onychomycosis in the general population. Oral agents are absorbed via the circulation through the nail bed and take nearly 7 days to reach minimal inhibitory concentration (MIC). Once administration of the drug is discontinued, it can remain active in the nail for up to 90 days, and the nail does not need to be completely clear before the medication is stopped.

Griseofulvin was the standard oral therapy for onychomycosis for more than 30 years. However, it has a narrow therapeutic window and significant adverse reactions. It also has several interactions with other drugs and is active only against dermatophytes, with a cure rate of less than 40%. For these reasons, it is rarely used today to treat onychomycosis.

The imidazole class of medications is active against most of the organisms that cause onychomycosis. Ketoconazole is slightly more efficacious than Griseofulvin but also has many adverse effects and drug interactions.

It is rarely used to treat onychomycosis today. Fluconazole, 300mg once a week for 6 months, is more efficacious and has been shown to be safe. Itraconazole, a triazole antifungal, binds more specifically to fungal cytochrome P-450 than other

azoles, reducing the incidence of side effects.

It is active against dermatophytes *Candida* and *Aspergillus* but not *Scytalidium*, a mold. Because of the high cost of Itraconazole, a pulse regiment has been formulated and tested. Pulse treatment involves using 200 mg twice daily for 1 week during each of 2 months in fingernails and 3 months in toenails. Pulse therapy has been reported to be just as effective as continuous therapy with fewer adverse events and half the cost. Terbinafine, 250 mg once daily for 3 months, has been shown to achieve a mycological cure rate of 82% in toenail onychomycosis and 71% in fingernail onychomycosis.

Table No.1: Treatment and mycological cure rates

Treatment	Mycological cure rates
Terbinafine(continuous)	76 %(±4%)
Itraconazole(pulse-dose)	63 %(±7%)
Itraconazole(continuous)	59 %(±5%)
Griseofulvin	40 %(±6%)
Fluconazole	48 %(±5%)
Miconazole	53.4 %(±7%)

Disadvantages of oral therapy

Oral therapy is followed by some disadvantages such as drug interactions, contraindications, side effects, high cost of medication, and a long duration of treatment. Moreover, systemic use of azoles can be linked to hepatotoxicity, especially during prolonged use.

1.9.3 Topical therapy

There are three classes of topical antifungal creams: polyenes (e.g.Nystatin), imidazoles (e.g.Clotrimazole), and allylamines- benzylamines (e.g.Terbinafine).

All three are active against *Candida*, but only imidazoles and allylamines benzylamines are active against dermatophytes. In general, topical therapy is not adequate for clearing nail infections, probably because of inadequate penetration of the medication into the affected tissues and nail bed. The exception to this is superficial white onychomycosis, which is easily treated with a topical agent because the organism grows on the upper nail plate rather than in the nail bed. Antifungal nail lacquers are available for treating onychomycosis and penetrate the nail better than creams and gels. One lacquer contains the active ingredient Amorolfine, which is in a new class of antifungals, the morpholines. Another lacquer contains Ciclopirox, which has a broader spectrum of activity. Nail lacquers are applied daily for 48 weeks and once-weekly removal with nail polish remover is required.

Demerits of topical therapy

- They should be reserved for mild distal disease in up to two nails, or for superficial white onychomycosis, or where there are contra-indications to systemic therapy.
- Treatment should be given daily for six months to one year
- Topical therapy often fails due to poor penetration through nail plate and provide less contact time

To overcome the disadvantages of conventional topical drug delivery system, a noval formulation called medicated nail lacquers has been formulated and was reported to be a potential drug delivery system for the treatment of Onychomycosis.
The Food and Drug Administration (FDA) approved Ciclopirox nail lacquer for the treatment of mild to moderate onychomycosis caused by *Tinea rubrum* without involvement of the lunula [20, 21].

1.10 NAIL LACQUER[3, 19, 20, 21]

Nail polish or nail varnish is applied to human fingernails or toenails to decorate and/or protect the nail plate. Conventional nail lacquers have been used as cosmetics since a long time for beautification and protection of nails.

Topical nail preparations like lacquers, enamel and varnish are an integral part of today's beauty treatments. It protects the nail plate, but more importantly it enhances their beauty, imparting color and luster.

A model nail lacquer should have the following properties:

- It should be harmless to skin and nails.
- It should be convenient and easy to apply.
- It should be stable on storage
- It should form a satisfactory film on nails.

To achieve satisfactory film it should have the following characteristics:

- It should have good wetting and flow properties so that the film formed is even.
- It should have uniform colour.
- It should have good gloss.
- It should have good adhesive properties.
- It should have sufficient flexibility so that it does not crack or become brittle.
- It should have sufficient hard surface which is resistant to impact and scratch.
- It should have reasonable drying time (1-2 minutes) without developing bloom.
- It should be able to maintain the above-mentioned properties for a reasonable time (about 1 week).

Constituents of nail lacquer

The basic nail varnish consists of solvents, film forming polymers, resins which enable the film to adhere to nail plate and convey shining to the film, plasticizers which give flexibility and durability to the film, colouring agents and suspending agents.

a. Film formers

A number of film forming substances have been suggested for nail enamels. These include nitrocellulose, cellulose acetate, cellulose acetate butylate, ethyl cellulose, vinyl polymers and various polymers of methacrylate.

b. Resins

Resins impart adhesion and improve gloss. Commonly used resins are Santolite MHP and Santolite MS 80%. They are claimed to increase moisture

resistance. They are soluble in majority of solvents.

c. Plasticizers

Plasticizers impart flexibility and adhesive properties to the film. There are two types of plasticizers, solvent and non-solvent plasticizers. The amount of plasticizers which can be used in nail lacquers varies widely and may vary from 25% to 50% of film former. The amount depends on flexibility of film required. Dibutyl phthalate is most widely used plasticizer.

d. Solvents

Although evaporation characteristics are of prime importance in nail lacquers, but rapid rate of evaporation causes a poor flow of enamel resulting in uneven and streaky application. Solvents are generally classified according to their boiling points.

1) Low boiling point solvents: e.g. Ethyl ether, Acetone, Ethyl alcohol etc.

2) Medium boiling point solvents: e.g. N-Butyl acetate, n-Butyl alcohol etc.

3) High boiling point solvents: eg. Ethyl lactate. Alcohols, particularly ethyl, isopropyl and butyl are very efficient diluents.

e. Pigments

Pigments used in nail enamels should have the same properties as required in other cosmetics. commonly used pigments are titanium dioxide, yellow iron oxide, red iron oxide, etc.

f. Suspending agents

Insoluble pigments and iridescent materials have tendency to settle. Therefore to avoid this suspending agents such as colloidal clays like bentonite can be used.

In recent past, medicated lacquers specially designed for the nail diseases, strike the formulation field. Nail diseases like onychomycosis, nail psoriasis, yellow nail syndrome, paronychia and many more, being cured successfully using medicated lacquers. This avoids the oral toxicity of anti-fungal drugs and provides longer contact time at the site of action. This systemic review covers the anatomy of a human nail, diseases related to nail plate, the formulations designed for nail application and some

techniques used to enhance the topical bioavailability of the drugs across the nail, latest trends in drug delivery across the nail.

Nail lacquer can be used as a drug delivery system for the drugs that exhibit poor oral bioavailability. The topical formulations conventionally used in dermatology (creams, oil-based lotions, powders) are not specifically adapted to the nail since they are readily removed by rubbing, whipping, and washing; and their impermeance at the site of application readily accounts for their inefficacy.

Medicated nail lacquers are formulations that are used for ungual drug delivery system for maximal antifungal efficacy. It has been reported that the film on the nail surface acts as a drug depot that permits optimized and sustained diffusion across the nail and leads to continuous penetration of active principle to high tissue concentration required for the efficacy for the treatment of onychomycosis.

The present invention relates to a formulation for treating fungal infections. More specifically, this formulation is a topical formulation for use on fingernails and toenails. Many people have fingernails or toenails with fungus underneath. Still others have nails that are extremely thick even approaching approximately one inch in thickness. Still others have yellowed or discolored nails.

Some have combinations of the above-mentioned conditions. Some medications available for treating these unsightly conditions are not able to kill fungal infections underneath the nail because they are not able to penetrate the nail.

Still other medications cause the nail to become brittle. In addition, other medications simply do not work. Therefore, many people are unable to remove these unsightly conditions. In order to overcome the disadvantages of medications currently available, a formulation that is able to penetrate the nail to kill fungus without permanently damaging the nail is needed.

This formulation should be able to be applied topically. The human nail is an excellent barrier against the ingress of foreign material, but as a consequence it also prevents effective topical treatment of ungula disorders such as onychomycosis.

When infection resides in the nail plate, nail bed, or both, therapeutic antimicrobial drug concentrations must be achieved in the nail bed for treatment to be effective. However, this is difficult to achieve because the permeation of agents into the nail is low.

Permeation occurs via passive diffusion with the rate determined by the physicochemical properties of the compound. Unfortunately, the most efficacious treatments for nail disorders do not penetrate the nail plate in sufficient amounts to be clinically effective.

The common approach for enhancing nail drug delivery has been to use keratolytic and thiolytic agents. These agents are known to increase the permeability of nail matrix by chemical modification of keratin. However, their permeability enhancement potential is limited by the factors like penetrability of enhancer and the duration of its presence in the nail matrix might significantly influence the chemical modification of keratin.

Topical monotherapy is considered less efficient in treating nail disorders such as onychomycosis due to poor trans-nail bioavailability of drugs.

1.11 ABSORPTION THROUGH NAIL [22]

Nail plate is approximately 0.25–0.6 mm, which is approximately 100-fold thicker than the stratum corneum. In contrast to the stratum corneum, the nail plate behaves like a concentrated hydrogel rather than a lipophilic membrane. Hydration can affect the effective pore size of hydrogel and thus the transungual transport. Hydrated human nail plates behave like a hydrogel of high ionic strength to the polar & semi polar alcohols [6]. Moreover the nail is primarily enriched with highly disulfide-linked keratin. The nail selects for the penetration of small, hydrophilic molecules[3]. Most pharmaceutical agents are large and highly lipophilic, and are therefore unable to diffuse across the nail at therapeutic concentrations. Lipophilic vehicles and especially nail lacquers are more appropriate for topical application on the nail than aqueous systems because of their better adhesion[23]. Penetration through the nail plate follows first order kinetics after a lag-time of 400 hours. The course of penetration initially is membrane-controlled and later becomes a matrix-controlled process because of the membrane's greater permeability.

1.12 FACTORS INFLUENCING UNGUAL DRUG DELIVERY [23, 24]

- Molecular size of diffusing molecule
- Hydrophilicity/lipophilicity of diffusing molecule
- Nature of vehicle
- pH of vehicle and solute charge
- Presence of an intact dorsal layer
- Binding of drug to keratin and other nail constituents
- Presence of disease can alter the properties of nail
- Thickness of the nail

a. Molecular size of diffusing molecule

Molecular size is inversely proportional to the penetration into the nail plate. It is harder for larger molecules to diffuse through the keratin network. The molecular weight of most of the antifungal agents is > 300 Dalton. Hence these drugs will have difficulty penetrating the nail plate which can be a reason for the poor clinical efficacy.

b. Hydrophilicity/Lipophilicity of diffusing molecule

On increasing lipophilicity of the diffusing alcohol molecule the permeability coefficient reduces until a certain point beyond which further increase in lipophilicity results in increased permeation. However, with the exception of methanol, the permeability coefficient of neat alcohols (absence of water) was approximately 5 times smaller than the permeability coefficient of diluted alcohols. When an aqueous formulation is used; the nail swells as water is absorbed into the nail plates. As a result the keratin network expands leading to the formation of larger pores through which diffusing molecules can permeate more easily.

c. Nature of vehicle

As the nail absorbs water, it swells. This swelling results in

increased distance between the keratin fibers and leads to the formation of larger pores through which permeating molecules can diffuse. This is due to hydrogel property of nail plate. On replacing water with a non-polar solvent, that does not hydrate the nail; the drug permeation into the nail plate is expected to reduce. Aqueous vehicles are not effective as lipophilic vehicles for topical application because they are easily washed / wiped off and do not adhere as well to the nail plate.

d. pH of vehicle and solute charge

Uncharged species tend to permeate better than the charged ones. The pH of aqueous formulations influences the ionization of weakly acidic/basic drugs,

which in turn has an effect on:

- ❖ the drug's hydrophilicity / hydrophobicity,
- ❖ solubility in the drug formulation,
- ❖ solubility in the nail plate and
- ❖ Its interactions with the keratin matrix.

e. Presence of an intact dorsal layer

It is generally recognized that the very thin dorsal layer with its overlapping cells represents the greatest barrier to the drug penetration across the nail plate. If this layer is partially or totally removed e.g., by debridement or chemical etching with 30-40% phosphoric acid or use of keratinolytic enzymes, then drug permeability increases.

f.Binding of the drug to keratin and other nail constituents

pH of keratin ranges around 5 and therefore is positively and negatively charged at pH below and above this value. It might bind or repel molecules depending on their charge. This can be one of the reasons of lower nail permeability of ionic

compounds. It has been shown that a number of drugs including Terbinafine and Amorolfine bind strongly to keratin, and can influence their respective antifungal activity.

g. Presence of disease can alter the properties of nail[24]

Various nail conditions affect the nail very adversely. Conditions such as Onychogryposis is characterized by a thickened nail plate, Onychatrophia is an atrophy or wasting away of the nail plate, Tinea unguis of the nails, is characterized by nail thickening, deformity, and eventually results in nail plate loss. Pseudomonas can even cause the nail plate to lift from the nail bed.

h.Thickness of the nail - Greater the thickness, lower is the permeation of the drug through the nail plate.

1.13 PENETRATION THROUGH NAILS[23]

The penetration of drug into nail is quite difficult due to various factors such as the molecular size of the drug, hydrophilicity, pH, solute charge. Consequently researches are currently being undertaken to design novel in vitro methods to assess the ability of compounds to penetrate the nail plate [**13**]**.** The goal of topical therapy for Onychomycosis is drug penetration into deep nail stratums at amounts above the minimal inhibitory concentration (MIC). Effective penetration still remains challenging as the nail is composed of approximately 25 layers of tightly bound keratinized cells which in comparison to stratum corneum is 100- fold thicker.

Different methods developed so far are:

A. Chemical means
B. Mechanical means
C. Physical means

A. Chemical methods to enhance nail penetration

Chemically, drug permeation into the nail plate can be assisted by breaking the physical and chemical bonds responsible for the stability of nail keratin. This would destabilize the keratin, compromise the integrity of the nail barrier and allow penetration of drug molecules.

Wang and Sun (1998), identified the disulphide, peptide, hydrogen and polar bonds in keratin that could potentially be targeted by chemical enhancers such as:

1) Nail softening agents or Keratolytic enhancers

Keratolytic agents such as (papain, urea, and salicylic acid) enhance the permeability of three imidazole antifungal drugs (Miconazole, Ketoconazole, and Itraconazole) Urea and salicylic acid hydrate and soften nail plates.

Urea and salicylic acid also damage the surface of nail plates, resulting in a fractured surface. Effects of the physical enhancers were penetrant specific, but the use of a reducing agent followed by an oxidizing agent (Urea, H_2O_2) dramatically improved human nail penetration while reversing the application order of the physical enhancers was only mildly effective. Both nail physical enhancers are likely to function via disruption of keratin disulphide bonds and the associated formation of pores that provide more 'open' drug transport channels

2) Compounds containing sulfhydryl groups

Compounds which contain sulfhydryl (SH) groups such as acetyl cysteine, cysteine, mercaptoethanol can reduce, thus cleave the disulphide bonds in nail proteins, as shown in the reaction sequence below:

Nail-S-S-Nail + R-SH → 2 Nail – SH + R-S-S-R

R represents a sulfydryl-containing compound.

However, post-treatment barrier integrity studies demonstrated that changes induced in the nail keratin matrix by these effective chemical modifiers were irreversible. It is believed that these enhancers act by breaking disulphide bonds, which

are responsible for nail integrity thus producing structural changes in the nail plate.

3) Keratinolytic enzymes

Due to an abundance of keratin filaments, keratinic tissues like the Subcutaneous are effectively hydrolyzed by keratinase. It is hypothesized that keratinolytic enzymes may hydrolyze nail keratins, thereby weakening the nail barrier and enhancing ungual drug permeation. Keratinase act on both the intercellular matrix that holds the cells of the nail plate together and the dorsal nail corneocytes by corroding their surface.

B. Mechanical means involves

1) Nail abrasion

Nail abrasion thins the nail plate, decreasing the fungal mass of onychomycosis and exposing the infected nail bed.

2) Nail avulsion

Total nail avulsion and partial nail avulsion involve surgical removal of the entire nail plate or partial removal of the affected nail plate, and under local anesthesia. Keratolytic agents such as urea and salicylic acid soften the nail plate for avulsion. Urea or a combination of Urea and Salicylic acid has been used for nonsurgical avulsion.

C. **Physical means** of enhancing drug permeation involves the use of agents that by delipidization or fluidization of the intracellular lipids in the nail plate can help in drug permeation.

Some of the approaches have been used to resolve these barriers to drug delivery include:

1) Iontophoresis

Iontophoresis involves delivery of a compound across a membrane using an electric field (electromotive force). Drug diffusion through the hydrated keratin of a nail may be enhanced by iontophoresis.

2) Electroporation

Electroporation is a method in which, with the application of an electric pulse of about 100–1,000 V/cm creates transient aqueous pores in the lipid bilayers making the solute particles permeable through it.

3) Micro needle enhanced delivery systems

Method using arrays of microscopic needles to open pores in the SC directly to the skin capillaries; also has the advantage of being too short to stimulate the pain fibers, thus facilitating drug permeation.

4) Etching

"Etching" results from surface-modifying chemical (e.g. Phosphoric acid) exposure, resulting in formation of profuse micro porosities.

Presence of micro porosities improves "interpenetration and bonding of a polymeric delivery system and facilitation of interdiffusion of a therapeutic agent."

5) Carbon dioxide laser

CO2 laser may result in positive. Here involves penetrating the nail plate with CO2 laser beam. This method is followed with daily topical antifungal treatment, penetrating laser-induced puncture holes.

6) Hydration and occlusion

Hydration may increase the pore size of nail matrix, enhancing transungual penetration. Additionally, hydrated nails are more elastic and permeable. Decreases in transonychial water loss, ceramide concentration, and water binding capacity may result from Onychomycosis. Occlusion may resolve these changes via reconstitution of water and lipid homeostatisis in dystrophic nails.

Other physical penetration enhancements - Lasers, Phonophoresis, Ultraviolet light.

Need of the study:

Onychomycosis is a chronic condition which requires long duration of treatment. Oral therapies are accompanied by systemic side effect, while topical therapy are limited by low permeation and restrictive barrier property of nail plate. Medicated nail lacquer after application leaves an occlusive film over the nail, which act as a drug depot from which sustained release of antifungal is provided for entire duration of therapy. Therefore in the present study an attempt to formulate an antifungal agent as a nail lacquer formulation has been tried out. Although Miconazole has been a widely used as antifungal, no work has been reported to improve the permeation of Miconazole nitrate from a nail lacquer formulation. The purpose of this study is to explore the potential of an ideal permeation enhancer in improving penetration of drug across nail plate and whereby could result in an enhancement of bioavailability of antifungal drug.

2. AIM AND OBJECTIVE

Onychomycosis (tineaunguium) is a fungal infection of the nail bed or nail plate that accounts for approximately 50% of all nail diseases and is the most common disorder in adults. Onychomycosis though rarely life threatening, they can be very painful, discomfortable and disfiguring for the sufferer and may produce serious physical and occupational limitations, psychological and emotional effects, and affect quality of life. Deformed nails can lead to surrounding tissue damage and may promote secondary bacterial infection. Known methods for the treatment fall into three categories: 1. Removal of all or part of the affected nails 2.Oral / systemic therapy 3.Topical/Ungual therapy. Creams, ointments, gels, solutions, lotions, foams, pastes etc are available dosage forms for topical delivery. Topical or oral therapy of nail diseases are also limited by poor permeability of the nail plate, oral toxicity of antifungal agents and provide longer contact time to the site of action. In recent past, medicated lacquers specially designed for nail diseases. The treatment of onychomycosis is a challenging task because of unique barrier properties of the nail plate which hampers the passage of antifungal drugs in a concentration required to eradicate the deeply seated causative fungi in the nail bed. In present investigation, an attempt will be done to explore the potential of **2-hydroxypropyl-β-cyclodextrin** (2-HP-β-CD) as an effective and nail friendly transungual drug permeation enhancer for **Miconazole nitrate**, a poorly water soluble drug. **2-HP-β-CD** is expected to improve the hydration of nail plates and thereby increasing solubility of **Miconazole nitrate** which could further enhance its permeation across nail plate.

2.1 Objective of the study

The purpose of the present investigation is to formulate and evaluate an antifungal nail lacquer for treatment of onychomycosis. A nail lacquer will be formulated, consisting of antifungal drug Miconazole nitrate, film forming polymers like nitrocellulose, plasticizer like propylene glycol and other required additives. An attempt will be done to enhance the transungual drug permeation of **Miconazole nitrate** using various permeation enhancers.

2.2 Plan of work

A.Pre formulation studies

- **Physicochemical parameters**
  - a) Solubility profile
  - b) Melting point determination
- **Analytical method**
  - a) Determination of λ_{max}
  - b) Development of standard curve of Miconazole nitrate
- **Determination of compatibility of drug with polymer**
  - a) By FTIR spectroscopy

B.**Formulation of nail lacquer and optimization of permeation enhancer**

C. Evaluation of lacquers

- Non -volatile content
- Drying rate & film formation
- Smoothness to flow
- Gloss
- Viscosity
- Adhesion
- Percentage drug content determination
- In vitro diffusion study
- In vitro ungual permeation studies
- Anti- microbiological studies
- Stability studies as per ICH guidelines

3. REVIEW OF LITERATURE

Tandel Amruta1 et al, 2012, in their research studied the Transungual permeation of the voriconazole nail lacquer against Trichophyton rubrum. The purpose of study was to determine amount of voriconazole permeating through the nail plate from the nail lacquer formulation containing permeation enhancer. The permeability studies were performed on avulsed human cadaver nail plates using modified Franz diffusion apparatus containing phosphate buffer saline in the acceptor chamber. The addition of thioglycolic acid, 5% improved the permeability of the drug by 0.7 as the enhancement factor[25]

A N Merekar et al, 2012 formulated a medicated nail lacquer for preungual drug delivery. Enalapril Maleate was chosen as the model drug, and the formulations were prepared with and without polymer Eudargit RL 100 within the concentration range of 1% to 5% (w/v) in the polymeric system. Then, these lacquers were compared for glossiness, film formation, drying rate, smoothness of flow, and nonvolatile content. The in vitro studies were preformed on the artificial membrane in solvent A (phosphate buffer, pH 7.4; and methanol, AR grade, in the ratio of 4:1). The result obtained indicated that the nail lacquer formulation showed good release of the drug. Thus nail lacquers can be used as a successful tool for targeted drug delivery for hypertension.[26]

Gupchup GV et al, 2010, has reviewed the Structural characteristics and permeability properties of the human nail. He reported that nail is primarily composed of a highly cross-linked keratin network that contains several disulfide linkages. Compounds containing sulfhydryl groups in conjunction with keratolytic agents can significantly enhance drug penetration. Such sulfhydryl compounds are thought to reduce the disulfide linkages in the nail keratin matrix.[1]

S. Hadzidedic et al, 2010, in their article, Characterization of antifungal nail lacquer formulations containing fluconazole, developed six formulations of nail lacquer containing 0.9% (w/v) fluconazole, Eudargit RS 100 and acetone. The formulations contain ingredients like di-butyl phthalate, polyethylene glycol 400 or propylene glycol as plasticizers in two different concentrations. we characterized the developed formulations with regard to the drying time, fineness of formed film, fluconazole assay and viscosity.[27]

R.Shireesh Kumar et al, 2010, reported that Transungual delivery of ketoconazole from the nail lacquer for topical therapy of nail disease is limited by poor bioavailability of nail plate. They made various trials with permeation enhancers like cysteine, thioglycolic acid, urea, hydrogen peroxide and found an increase in permeability of applied ketoconazole drug.[20]

Ghannoum MA et al, 2010 determined the efficacy of different Terbinafine hydrochloride nail solutions (TNS) formulated with/without dodecyl-2-N,N-dimethyl amino propionate hydrochloride (DDAIP HCl). TNS containing 1%, 5% and 10% Terbinafine hydrochloride formulated with and without DDAIP HCl demonstrated high antifungal efficacy[27] Venjnovic I et al, 2010 determined the amount of Terbinafine hydrochloride penetration through the human nail plates from the liquid formulations containing enhancers hydrophobins A-C in the concentration of 0.1% (w/v). The used reference solution contained 10% (w/v) of Terbinafine in 60% (v/v) ethanol/water without enhancer. All tested hydrophobins facilitated terbinafine permeation after 10 days of permeation experiment; however one of them achieved an outstanding enhancement factor of 13.05 compared to the reference.[28]

Sigurgeirsson B et al, 2010 determined the efficacy of Amorolfine nail lacquer for the prophylaxis of Onychomycosis for a period of over 3 years. During the study Amorolfine was found safe and well tolerated during the study, with no treatment-related

adverse events.[29]

Monti D, et al, 2010 evaluated the antimycotic activity of a new water-soluble nail lacquer containing Ciclopirox (CPX/sol. Application of the Ciclopirox /sol nail lacquer allows rapid nail penetration of Ciclopirox, providing Ciclopirox levels sufficient to inhibit fungal growth for a prolonged period of time after application of lacquer dose.[30]

Sudaxshina M et al, 2010 reviewed the recent research into ungual drug delivery and also the drug delivery of nail lacquers through the nail and its penetration. Also reviewed the various factors affecting the drug uptake and penetration through the nail plate.[31]

Togni G et al, 2010, investigated the *in vitro* antifungal activity and *in vitro* an in vivo nail permeation of 8% Ciclopirox nail lacquer (P-3051). P-3051 is based on hydroxypropyl chitosan as film forming agent. P-3051 and the reference showed the same protective activity in experimental infections with strains of dermatophytes isolated from clinical samples.[32]

Bohn M et al, 2010, determined the Dermatopharmacology of Ciclopirox nail lacquer topical solution 8% in the treatment of Onychomycosis. Ciclopirox has been formulated in a nail lacquer delivery system. After evaporation of volatile solvents in the lacquer, the concentration of ciclopirox in the remaining lacquer film reaches approximately 35%, providing a high concentration gradient for penetration into the nail.[33]

Nadkar S et al, 2010, gave an OTC perspective of current trends of novel drug delivery systems. The global scenario and regulations in OTC category are introduced and the key potential delivery platforms and therapeutic categories in the OTC market are been highlighted.[34]

Roberts DT et al, 2010, gave the guidelines for treatment of Onychomycosis prepared for dermatologists on behalf of the British Association of Dermatologists. They present evidence-based guidance for treatment, with identification of the strength of evidence available at the time of preparation of the guidelines, and a brief overview of epidemiological aspects, diagnosis and investigation.[35]

Andrea Mayumi Koroishi et al, 2010, Antifungal activity and nail permeation of nail lacquer containing piper regnellii (miq.) c. cd. var. pallescens (c. dc.) yunck (piperaceae) leave extracts and derivatives, For this study the antidermatophyte activity of the extracts and derivates from leaves of Piper regnellii was analyzed. Nail lacquer containing the chloroform fraction showed good penetration through the nail as determined by photoacoustic spectroscopy. From in vitro studies it was observed that nail lacquer concentrations above 20 mg/mL prevented the growth of fungi, but concentrations up to 2.5 inhibited the growth. The specie P. regnellii showed great antifungal activity against T. rubrum, and nail lacquer containing its chloroform fraction has great potential to treat onychomycosis caused by this microorganisms.[36]

Alessandro et al, 2009, the pharmaceutical forms most widely investigated are 1% ciclopirox olamine cream and 8% ciclopirox acid nail lacquer. It penetrates into the deep layers of the skin, mucosal membranes and nail keratin, reaching concentrations exceeding the minimal fungicidal concentrations for most medically important fungi. Emphasis in this review is given to a ciclopirox medicated nail lacquer having good affinity to keratin and nail permeation. It has been found to have superior efficacy and safety to another commercially available formulation in the treatment of onychomycosis.[37]

B. V. Mitkari et al, 2007, Formulation and Evaluation of Topical Liposomal Gel for Fluconazole, In the present work statistical study for the formulation of liposomes for topical delivery of fluconazole using the factorial design approach was undertaken. Amount of phospholipid (PL 90H) and cholesterol (CH) were taken at three different levels and liposomes were prepared using film hydration technique. Liposomal dispersion and

gels were found to increase the skin permeation and deposition compared to control and marketed gel. Liposome dispersion and gel formulation were found to be stable for 60 days.[38]

R. P. Patel et al, 2009, Drug delivery across human nail, Topical nail preparations like lacqures, enamel ,varnishes are the integral part of today's beauty treatments. It protects the nail plate, but more importantly it enhances their beauty, imparting colour and luster. The basic nail varnish contains solvents, film forming agents, resins which enable the film adhere to nail plate and convey shining to the film, plasticizers give flexibility and durability to the film. Nail diseases like onychomycosis, nail psoriasis, yellow nail syndrome, paronychia and many more , being crushed successfully using medicated nail lacquers.[3]

Tulli A et al, 1988, the treatment of onychomycosis with a new form of tioconazole, the difficulties encountered in the treatment of onychomycosis are primarily related to the necessity of prolonged systemic therapy. Many of these difficulties could, then, be avoided by the use of an effective local treatment. The present study compared the effectiveness and tolerability of two topical ungual preparations: a 28% solution of tioconazole and a 2% tincture of Miconazole. The therapeutic results and tolerability of both preparations were found to be satisfactory. The tioconazole preparation proved to be slightly more effective although the difference was not statistically significant.[39]

Scher RK et al, 1998, Once-weekly fluconazole (150, 300, or 450 mg) in the treatment of distal subungual onychomycosis of the toenail, Onychomycosis is a prevalent infection of the nail caused primarily by dermatophytes. Fluconazole is active in vitro against the most common pathogens of onychomycosis, penetrates into the nail bed, and is clinically effective in the treatment of a wide variety of superficial fungal infections.[40]

B.Bentley-phillps, 1982, The treatment of onychomycosis with miconazole tincture,Treatment of onychomy.cosis is usually unsatisfactory. A small clinical trial using a solution of miconazole (Daktarin; Janssen) in alcohol was undertaken to assess its potential in this difficult condition. Ten unselected patients completed the 32-week trial and the results, irrespective of whether the infecting organism was fungal or monilial, are recorded. A much larger and controlled trial is essential before firm conclusions may be drawn, but the results, particularly in Candida infections, are encouraging.[41]

4. DRUG AND POLYMER PROFILE

4.1 Miconazole Nitrate[42, 43]

Table No.2:Properties of Miconazole nitrate

Proprietary name	Desenex, Monistat, Zeasorb-AF
IUPAC Name	(RS)-1-(2-(2,4-Dichlorobenzyloxy)-2-(2,4dichlorophenyl)ethyl)-1H- imidazole
Molecular formula	$C_{18}H_{14}Cl_4N_2O$
Molecular weight	416.127 g/mol
CAS No	22961–47–8
Melting Point	159-163 °C

Structure

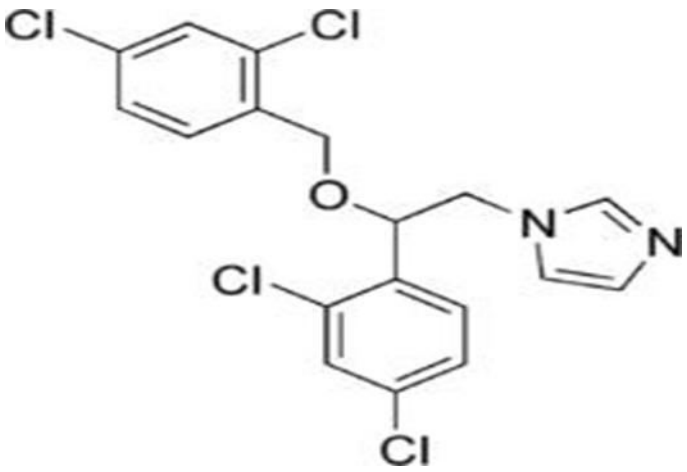

Figure 4: Structure of Miconazole nitrate

Description : The drug was found to be white crystalline powder with slight characteristic odour

Solubility of Miconazole : Soluble in methanol, Ethanol, Acetone

Mechanism of action

Miconazole interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents.

Miconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis.

Pharmacokinetics

Oral absorption of Miconazole (Nitrate) is found to be 20%. Volume of distribution is found to be 20 l/kg and plasma protein binding is 92 %. And metabolism is reported Hepatic. Renal Excretion accounts for 20 % and plasma half- life is 24.1 hr.

Dose

Adult Dosage : 2% for Ophthalmic and topical, 250.000 mg for PO

Paedriatic Dosage : 0.330 to 0.500 mg/kg as IV Infusion, 2.000 % for topical

Drug class : Anti -fungal

4.2 POLYMER PROFILE

4.2.1. Nitrocellulose [44, 45]

Table No.3: Properties of Nitrocellulose

Synonyms	Cellulose nitrate, flash paper, flash cotton, flash string, guncotton, Celex,Celloidin, Cellulose Tetranitrate, Kodak LR 115, Paralodion, Pyralin, Proxylin and Xyloidin
Empirical formula	$(C_6H_8(NO_2)_2O_5)n$
Molecular Mass	Variable
CAS Number	9004-70-0
Description	Yellowish white cotton-like filament
Melting point	160-170^{0}C(ignites)
Flash point	4.4^{0}C
Solubility	Good solubility in alcohol-type solvents,water-30 or 35%
Nitrogen Content	10.6-11.3%
Viscosity Value	350 - 450 mPa.s
Relative Density	0.8097g/cm^3

Structure

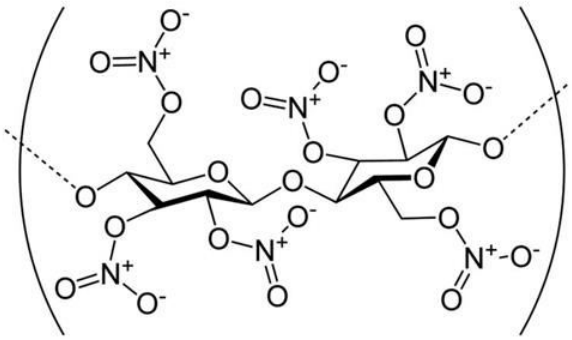

Figure 5: Structure of Nitrocellulose

Production

4-5gm of cellulose base (cotton) is added to concentrated sulfuric acid and 70% nitric acid (50:25ml) cooled to 5-10 °C to give cellulose trinitrate. Then cotton was removed and washed in cold water and solution of remove all acid residues. It was then slowly dried at a temperature. The process uses nitric acid to convert cellulose into cellulose nitrate and water.

$$3HNO_3 + C_6H_{10}O_5 \rightarrow C_6H_7(NO_2)3O_5 + 3H_2O$$

Hazards identification

Label precautionary statements: flammable (usa), highly Flammable (eu). Keep away from sources of ignition—no Smoking. Take precautionary measures against static discharges. Wear suitable gloves and eye/face protection.

Incompatibilities

Strong acids, strong bases, cellulose and its derivatives may react vigorously with calcium oxide, bleaching powder, perchlorates, perchloric acid, sodium chlorate, flourine, nitric acid and sodium nitrate.

Uses

Propellant or low-order explosive, film base in photograph, X-ray films and motion picture films.

Storage

Storing it wet or in oil, avoid direct sunlight.

4.2.2 Ethyl Cellulose[46]

Table No.4: Properties of Ethyl cellulose

Nonproprietary Names	BP: Ethyl cellulose, PhEur: Ethylcellulosum, USPNF:Ethylcellulose
Synonyms	Aquacoat ECD; Aqualon; Ethocel; Surelease.
Molecular formula	$(C_{12}H_{23}O_5)n$
CAS Number	9004-57-3
Description	Ethyl cellulose is a tasteless, free-flowing, white to light tan- coloured powder
Density (Bulk)	0.4 g/cm^3
Specific Gravity	1.12-1.15 g/cm^3
Melting Point	158-162^oC.
Moisture content	ethyl cellulose absorbs very little water from humid air or during immersion, and that small amount evaporates readily

Solubility

Insoluble in water & glycerin, but soluble in varying degree in certain organic solvents, depending upon the ethoxyl content.

Functional Category:

Coating agent

Flavoring fixatives

Tablet binder & filler

Viscosity-increasing agent.

Applications in Pharmaceutical Formulation or Technology

Ethyl cellulose is widely used in oral and topical pharmaceutical formulations. Ethyl cellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the stability of a formulation. Ethyl cellulose, dissolved in an organic solvent or solvent mixture, can be used on its own to produce

water-insoluble films. Higher-viscosity ethyl cellulose grades tend to produce stronger and more durable films.. In topical formulations, ethyl cellulose is used as a thickening agent in creams, lotions, or gels, provided an appropriate solvent is used.

Structure

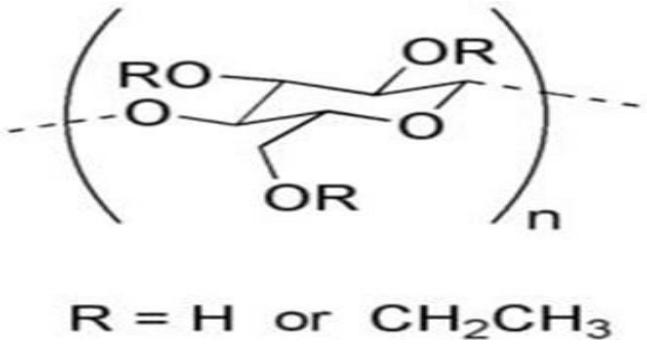

Figure 6: Structure of Ethyl cellulose

Stability and Storage Conditions

Ethyl cellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters. Ethyl cellulose should be stored at a temperature not exceeding 32°C in a dry area from all sources of heat. It should not be stored next to peroxides or other oxidizing agents.

4.2.3. 2- Hydroxypropyl -β-Cyclodextrin [46]

Structure

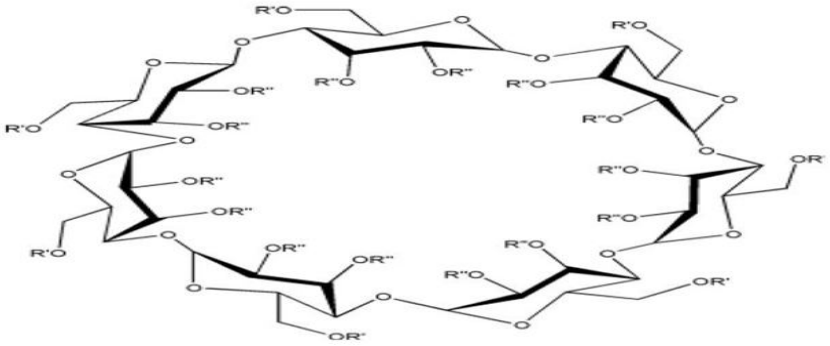

Figure 7: Structure of β-cyclodextrin (7 glucose units)

R′, R″ = H for 'natural' α-, β- and γ-cyclodextrins

R′, R″ = CH3 for methyl cyclodextrins

R′, R″ = CHOHCH3 for 2-hydroxyethyl cyclodextrins

R′, R″ = CH2CHOHCH3 for 2-hydroxypropyl cyclodextrins

Table No.5: Properties of 2- Hydroxypropyl -β-Cyclodextrin

Synonyms	Beta-cyclodextrin, ßCD, BCD, ß-Schardinger dextrin, cyclodextrin B.
Definition	A non-reducing cyclic saccharide consisting of seven alpha-1,4-linked D-glucopyranosyl units Manufactured by the action of cyclodextrin transglycolase on hydrolysed starch followed by purification of the ß-cyclodextrin; purification is by preparation of a ß-cyclodextrin/solvent inclusion compound followed by steam-stripping of the solvent before final purification.
Chemical names	Cycloheptaamylose
C.A.S. number	7585-39-9
Chemical formula	$(C_6H_{10}O_5)7$
Formula weight	1135.00
Description	Virtually odourless, slightly sweet tasting white or almost white crystalline solid
Solubility	B-cyclodextrin: soluble 1 in 200 parts of propylene glycol, 1 in 50 of water at 20°C, 1 in 20 at 50°C; practically insoluble in acetone, ethanol (95%), and methylene chloride.

Functional Uses : Encapsulation agent for food additives, flavoring and vitamins

- To increase water solubility of poorly soluble drugs
- To improve bioavailability of drugs
- To improve the organoleptic properties of drugs by masking bitter taste and nauseous taste.

4.2.4. Salicylic Acid[46]

Structure:

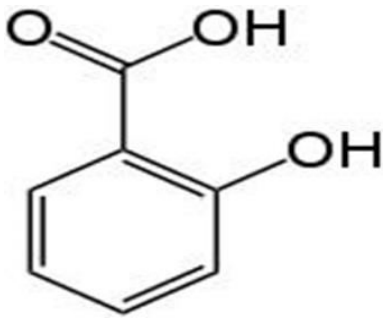

Figure 8: Structure of salicylic acid

Table No.6: properties of Salicylic Acid

Non-Proprietary Name	Acidum Salicylicum U. S. P
Synonym	2 hydroxy benzoic acid
Empirical formula	$C_6H_4(OH)COOH$
Molecular Mass	138.12 g mol−1
CAS Number	69-72-7
Solubility	Poorly soluble in water (2 g/L at 20 °C), freely soluble in alcohol
Density	1.443 g/cm^3
Melting Point	159.0 °C, 432 K, 318 °
Boiling Point	211 °C, 484 K, 412 °F (20 mmHg)

4.2.5. Propylene Glycol[46]

Structure

Figure 9: Structure of propylene glycol

Table No.7: Properties of Propylene Glycol

Non-proprietary name	BP :Propylene glycol, JP: Propylene glycol, PhEur: Propylene glycol,
Synonyms	α-propylene glycol, 1,2-propanediol, 1,2-Dihydroxypropane, methyl ethyl glycol(MEG), methylethylene glycol, PG, Sirlene, Dowfrost.
IUPAC name	propane-1,2-diol
Molecular formula	$C_3H_8O_2$
Molar mass	76.09 g/mol
CAS number	57-55-6
Description	Colorless, odorless, clear, viscous liquid with a faintly sweet taste.
Density	1.036g/cm³
Melting point	−59 °C (−74 °F)
Boiling point	188.2 °C (370.8 °F)
Solubility	Soluble in water, ether, ethanol, chloroform
Specific Gravity	1.036 (Water = 1)
Water/Oil Dist. Coeff.	The product is more soluble in water; log(oil/water) = -0.9
Viscosity (dynamic)	58.1-mPa s (58.1 CP) at 20°C.
Storage	Hygroscopic. Keep container tightly closed. Keep container in a Cool, well-ventilated area. Do not store above 23°C (73.4°F).

Uses

Propylene glycol has been used as a solvent, extractant, and preservative in a variety of parenteral and nonparenteral pharmaceutical formulations.

5. MATERIALS AND METHODS

5.1 .LIST OF MATERIALS USED

Table No.8: List of materials used

Sl.no	Materials used*	Sources
1	Miconazole nitrate	Yarrow chemicals, Mumbai
2	HP- β- CD	Yarrow chemicals, Mumbai
3	Ethyl cellulose	Kemphasol, Popatwadi, Mumbai
4	Nitro cellulose	Kemphasol, Popatwadi, Mumbai
5	Propylene glycol	Laboratory grade, Otto kemi, Mumbai
6	Salicylic acid	Laboratory grade, Nice chemicals Pvt. Ltd Cochin
7	Ethyl alcohol	Laboratory grade,Jiangsu Huaxi InternationalTrade Co.Ltd,China
8	Sodium hydroxide	Laboratory grade, Nice chemicals Pvt. Ltd Cochin
9	Potassium dihydrogen phosphate	Laboratory grade, Nice chemicals Pvt. Ltd Cochin

*All chemicals and solvents used were of Analytical grade.

5.2. LIST OF EQUIPMENT USED:

Table No.9: List of equipments used

Sl.no:	Equipments	Manufacturers
1	Electronic weighing balance	Shimadzu Corporation, Japan
2	FT-IR	Perkin Elmor, USA
3	Double beam UV spectrometer	Shimadzu Corporation, Japan
4	Magnetic stirrer	Remi Electrotechnic Ltd, Vasai India
5	Desiccator	Technico, Delhi
6	Franz diffusion cell	Murthys, Hyderbad
7	Hot air oven	Rotex instruments, B&C industries, Kerala
8	Screw gauge	Technico, Delhi
9	Tensile strength apparatus	Dept. of Pharmaceutics, APSC

5.3. PREFORMULATION STUDIES

5.3.1. Identification Drug

A) Solubility study[47]

Saturated solubility of Miconazole nitrate was prepared using 10 ml of distilled water/ ethanol/ acetone in 25 ml volumetric flasks in triplicate. Precaution was taken so that the drug remains in medium in excess. Then by using mechanical shaker, the flasks were shaken for 48 hours. The sampling was done on 24^{th} & 48^{th} hour. The sample withdrawn (1 ml after filtration) was diluted with appropriate medium and analyzed by using UV spectrophotometer at 223 nm.

B) Melting point determination[47]

Melting point of drug was determined by taking a small quantity of drug in a capillary tube sealed at one end and was placed in Thiel's melting point apparatus and temperature range at which the drug melted was noted. Average of triplicate readings was noted.

C) Determination of λ_{max}

100 mg of pure Miconazole nitrate was taken in a volumetric flask and dissolved in a little of phosphate buffer pH of 7.4 and volume made up to100ml. 1ml of the above solution was taken and further diluted to 100ml. The above solution scanned for maximum absorbance in double beam UV-Visible spectrophotometer in between the range of 400-200 nm against phosphate buffer pH 7.4 as the blank. Triplicate readings were taken and average was calculated.

5.3.2. ANALYTICAL METHODS

A) Preparation of Phosphate buffer solution[47]

Preparation of 0.2M Sodium hydroxide solution

8gm of sodium hydroxide was dissolved in sufficient quantity of distilled water in a 1000ml volumetric flask and volume was made up to 1000ml with distilled water.

Preparation of 0.2M potassium dihydrogen ortho phosphate solution

27.218gm of potassium dihydrogen orthophosphate was dissolved in sufficient quantity of distilled water in a 1000ml volumetric flask and volume was made up to 1000ml with distilled water.

Preparation of phosphate buffer solution of pH 7.4

50ml of potassium dihydrogen ortho phosphate solution was taken in a 200ml volumetric flask and 39.1ml of 0.2M sodium hydroxide solution was added and made up to 200ml with distilled water.

B) Preparation of standard stock solution & Calibration curve of Miconazole nitrate

100mg of Miconazole nitrate pure drug was accurately weighed and transferred into a 100ml volumetric flask. Then the volume was made up to 100ml with PBS of pH 7.4, to obtain standard stock solution of Miconazole nitrate, having concentration 1000mcg/ml.

From the above solution aliquots of 2ml, 4ml, 6ml, 8ml, 10ml was pipetted out into another 100ml volumetric flask and made up to 100ml with PSB of pH

7.4 to obtain a concentration range of 20µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml, and 100µg/ml solution. This solution was analyzed at 223nm by using UV- Visible spectrophotometer. A graph of concentration **Vs.** absorbance was plotted. Drug content estimation and diffusion studies were based on this calibration curve.

5 .3.3. Determination of drug- polymer compatibility

FT-IR spectral analysis of pure drug and polymer were carried out individually and as mixtures. The compatibility between Miconazole nitrate, nitrocellulose, 2-HP- β- CD, propylene glycol and prepared formulation were carried out in the ratio 1:1. The samples were placed FT-IR window after mixing and triturating with potassium bromide.

Table No. 10: Drug- polymer compatibility study

Composition	Ratio	25^0C±2 /60^0CRH	40^0C±2 /75^0C RH
Miconazolenitrate	100mg	6 Months	1 Month
Nitrocellulose	100mg	6 Months	1 Month
HP- β- CD	100mg	6 Months	1 Month
Propylene glycol	100mg	6 Months	1 Month
Miconazole+ nitrocellulose	1:1	6 Months	1 Month
Miconazole + HP- β- CD	1:1	6 Months	1 Month
Final Formulation	NA	6 Months	1 Month

5.4. FORMULATION STUDIES

5.4.1. Development of nail lacquer of Miconazole nitrate

A) Preparation of Nitrocellulose[48]

About 5gms of cellulose base (cotton) is added to 50ml concentrated sulfuric acid and 25ml 70% nitric acid mixture and cooled to 5-10 °C to give cellulose nitrate. Then cotton was removed and washed in cold water and with $NaHCO_3$ solution to remove all acid residues. It was then slowly dried at room temperature.

B) Optimization of Nitrocellulose film former

Table No.11: Optimization of nitrocellulose film former

Formulation code	Nitrocellulose (%w/v)	Plasticizers(%w/v)		Ethanol(ml)
		PG	Glycerin	10
NF1	2	10	….	10
NF2	4	10	….	10
NF3	6	10	….	10
NF4	8	10	….	10
NF5	2	….	10	10
NF6	4	….	10	10
NF7	6	….	10	10
NF8	8	….	10	10

Four different concentrations of nitrocellulose, 2%, 4%, 6%, 8%, were prepared using two different plasticizers, Propylene glycol and Glycerine at 10 % concentration as per **Table No.11**. The optimum concentration for film formation was determined by evaluating the thickness, tensile strength, folding endurance and water resistance.

C) Evaluations of nitrocellulose film

a) **Film thickness** [49,50,51]

The thickness of the film was measured by using screw gauge with a least count of 0.01 mm at different spots of the films. The thickness was measured at five different spots of the film and average was taken.

b) **Folding Endurance** [49]

Folding endurance of the films was determined by repeatedly folding a small strip of the film (approximately 2x2 cm) at the same place till it broke. The number of times film could be folded at the same place, without breaking gives the value of folding endurance.

c) **Tensile Strength** [50]

The instrument used to measure the tensile strength was designed in pharmaceutics laboratory especially for this project work. The instrument is a modification of chemical balance used in the normal laboratory as shown in **Figure 10**. One pan of the balance was replaced with one metallic plate having a hook for attaching the film. The equilibrium of the balance was adjusted by adding weight to the right pan of balance. The instrument was modified in such a way that the patch can be fixed up between two hooks of horizontal beams to hold the test film. A film of 2.5cm length was attached to one side hook of the balance and the other side hook was attached to plate fixed up to the pan as shown in the **Figure 10.**

Tensile Strength (T)

$$\mathbf{T = Mg / Bt\ Dynes/cm^2}$$

T= force at break/ initial cross-sectional area of sample.

M = mass in grams

g = acceleration due to gravity 9.8m/sec²

B = breadth of the specimen in cm

t = thickness of sample in cm.

Figure 10: Tensile strength apparatus

d) **Water resistance**[51]

This is the measure of the resistance towards water permeability of the film. This was done by applying a continuous film on a surface and immersing it in water. The weight before and after immersion was noted and increase in weight was calculated. Higher the increase in weight lowers the water resistance.

5.4.2 Formulation of nail lacquer

The formulation trials were done as per formula given in **Table No: 12**. The mixture of Miconazole nitrate and Nitrocellulose was dissolved in Ethyl alcohol in the required quantity using a magnetic stirrer at a constant speed. To above clear solution required quantity of 2-HP- β- CD , Salicylic acid, and propylene glycol were mixed thoroughly and made up to the volume to 100ml. The prepared nail lacquer was transferred to a narrow mouthed, plastic screw capped glass bottle.

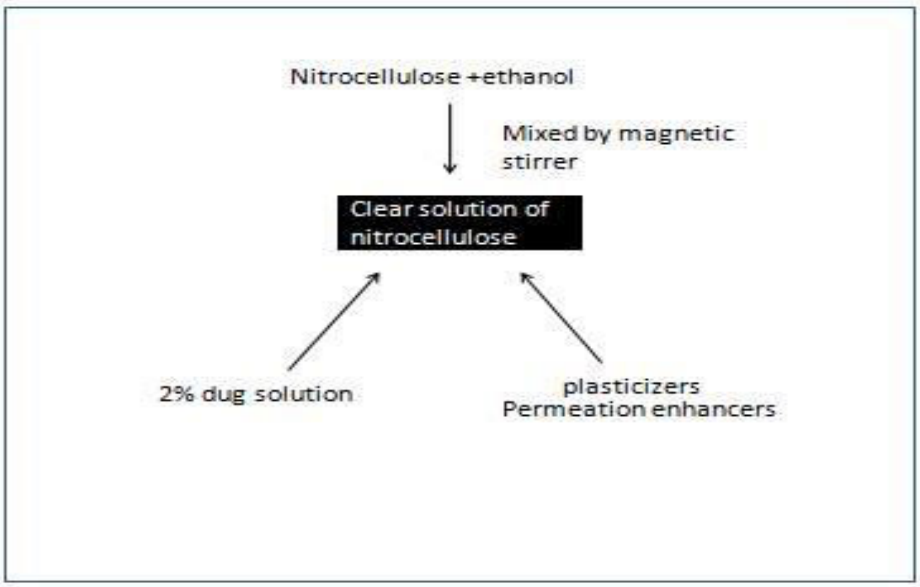

Figure 11: Schematic representation of preparation of nail lacquer

Table No.12: Formulation of Nail Lacquer

Ingredients (%)	**F0**	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**
Miconazole nitrate	2	2	2	2	2	2	2	2	2	2	2	2
Nitrocellulose	6	6	6	6	6	6	6	6	6	6	6	6
Salicylic acid		5	10	15	20	15	15	15	15	15	15	15
2-H- β-CD	…	…	…	…	…	5	7.5	10	10	10	10	10
Ethyl cellulose	…	…	…	…	…	…	…	…	0.25	0.50	0.75	1.00
Propylene Glycol	10	10	10	10	10	10	10	10	10	10	10	10
Ethanol qs	100	100	100	100	100	100	100	100	100	100	100	100

5.5 EVALUATION OF NAIL LACQUER[3, 20, 21, 22]

A) Nonvolatile content

10 ml of sample was taken in a petri dish and initial weights were recorded. The dish was placed in the oven at 105^0C for 1hr, the petri dish was removed, cooled and weighed. The difference in weights was recorded. Average of triplicate readings was noted.

B) Drying time

A film of sample was applied on a petri dish with the help of a brush. The time to form a dry-to- touch film was noted with the help of stop watch.

C) Smoothness to flow

The sample was poured from a height of 1.5 inches into a glass plate and spread on a glass plate and made to rise vertically and visually observed for smoothness of film.

D) Gloss

Sample of nail lacquer was applied over the nail and gloss was visually seen, compared with marketed cosmetic nail lacquer.

E) Viscosity

Viscosity was determined using Brookfield Viscometer, model LVF at room temperature using spindle no. 3 at 20 rpm.

F) Adhesion

There are no quantitative evaluation tools available to assess the medicinal nail lacquer at this time. Hence an equipment designed in the Pharmaceutics Lab has been used to determine the adhesive property of nail lacquer. The instrument is a modification of chemical balance used in the normal laboratory as shown in Fig.no: 10. One pan of the balance was replaced with two stainless steel plates. In between the plates a film of 4 cm^2 was prepared and adhered. The equilibrium of the balance was adjusted by adding a weight to the right pan of balance. The force required to pull away the plates is recorded and compared with a commercial cosmetic nail lacquer sample.

Force of Adhesion = Mass x Acceleration due to gravity

= Kilogram. meter/ second^2

= Newtons.meter/seconds2

Adhesive Strength = $\frac{\text{Force of Adhesion}}{\text{Surface area (m}^2\text{)}}$ (N)

G) Drug content estimation[49]

Nail lacquer equivalent to 200mg was dissolved in 50 ml phosphate buffer solution of pH 7.4. Then the solution was ultrasonicated for 15 mints. The resulting solution was filtered, made up to 100 ml with phosphate buffer solution of pH 7.4. From the above solution take 10ml and made up to 100ml with PBS of pH 7.4. Then the diluted solution was estimated spectrophotometrically at wavelength of 223 nm and determined the drug content.

H) Diffusion studies across artificial membrane [3, 20, 21]

Diffusion studies were performed by Franz diffusion cell using artificial membrane (cellophane) of 0.8µm. The membrane was soaked for 24hrs in solvent system and the receptor compartment was filled with solvent.

Nail lacquer equivalent to 200mg was applied evenly on the surface of the membrane.

The prepared membrane was mounted on the cell carefully to avoid entrapment of air bubbles under the membrane. The whole assembly was maintained at 37°C, and the speed of stirring was kept constant for 20hrs. The 5ml aliquot of drug sample was taken at time intervals of **2hr, 4hr, 6hr, 8hr, 10hr, 12hr, 16hr and 20hrs** and was replaced by the fresh solvent. Samples were analyzed by double-beam UV spectrophotometer as per method mentioned in drug content estimation. Each experiment was repeated thrice.

I) In vitro ungual permeation studies[49]

Hooves from freshly slaughtered cattle, free of adhering connective and cartilaginous tissue, were soaked in distilled water for 24hrs. Membranes of about 1mm thickness were cut from the distal part of hooves. In vitro permeation studies were carried out by using Franz diffusion cell, the hoof membrane was placed carefully on the cell. Then the nail lacquer equivalent to 200mg was applied evenly on the surface of the nail membrane. The receptor compartment was filled with solvent phosphate buffer solution of pH 7.4, and the whole assembly was maintained at 37°C with constant stirring for 48hrs. The 5ml aliquot of drug sample was taken after a time intervals of **2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48hrs** and was replaced by the fresh solvent. The drug analysis was done by using double-beam UV spectrophotometer at 223nm.

J) Determination of antimicrobial activity[49]

Candida albicans were employed for testing antifungal activity using the cup-plate method. The culture was maintained on sabouraud's agar slants. 20 ml of melted sabouraud's agar medium was inoculated with 72 hrs. old 0.2 ml suspension of *Candida albicans* in the Petri dish and allowed to set by keeping undisturbed for 15 mints.

The cups (10mm diameter) were punched in the Petri dish and filled with 0.05 ml of a solution of the sample. The plates were kept for diffusion at 40^0C for 1hr, and followed by incubation at 30^0 C for 48 hrs. After the completion of incubation period the zone of inhibition in millimeter were measured. Along with the test solution in each petri dish one cup was filled up with solvent, which act as control. The zone of inhibition was recorded and compared with control.

K) Stability study

Stability studies of nail lacquers were carried out as per ICH guidelines. Samples were stored at temperature of 25±2 ^{0}C/60 ± 5% RH for 6months and 40 ± 2^{o}C/75 ± 5% RH for 1 month. Then the samples were analyzed for non - volatile content, drying time, gloss, smoothness of flow, drug content and diffusion across artificial membrane.

6. RESULTS AND DISCUSSIONS

6.1. Results for Analytical study

6.1.1 Scanning of drug

Pure Miconazole nitrate sample was scanned using phosphate buffer solution (PBS) of pH 7.4 between 200nm to 400nm using UV visible spectrophotometer. The highest peak of Miconazole nitrate was obtained at 223nm (**Figure 12**) and thus the λ_{max} of Miconazole nitrate was fixed at 223nm and was used further spectrophotometric evaluations during the investigation.

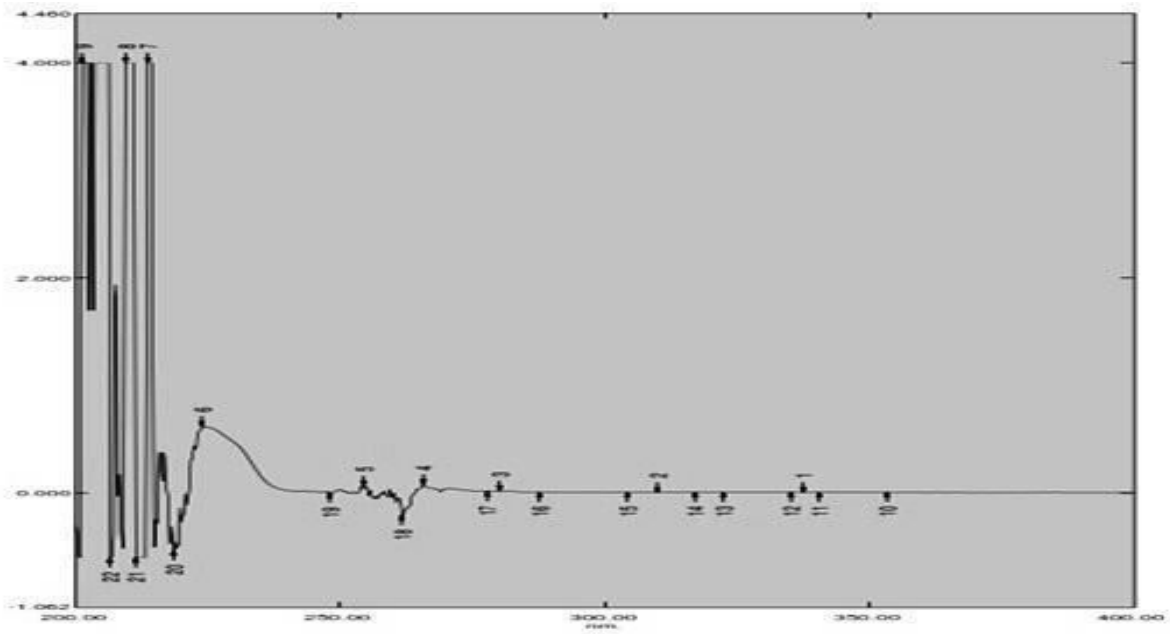

Figure 12: UV spectrum of Miconazole nitrate in phosphate buffer solution of pH 7.4

6.1.2 Standard curve for Miconazole nitrate in phosphate buffer of pH 7.4

Standard solutions of Miconazole nitrate in different concentrations (**Table No.13**) were prepared using PBS pH 7.4 and their absorption was measured at 223nm. Drug concentration Vs. absorbance was plotted in **Figure 13.**

Table No.13: Standard curve data for Miconazole nitrate in phosphate buffer of pH 7.4

Concentrations µg/ml	Absorbance at 223nm
0	0
20	0.124
40	0.245
60	0.364
80	0.487
100	0.609

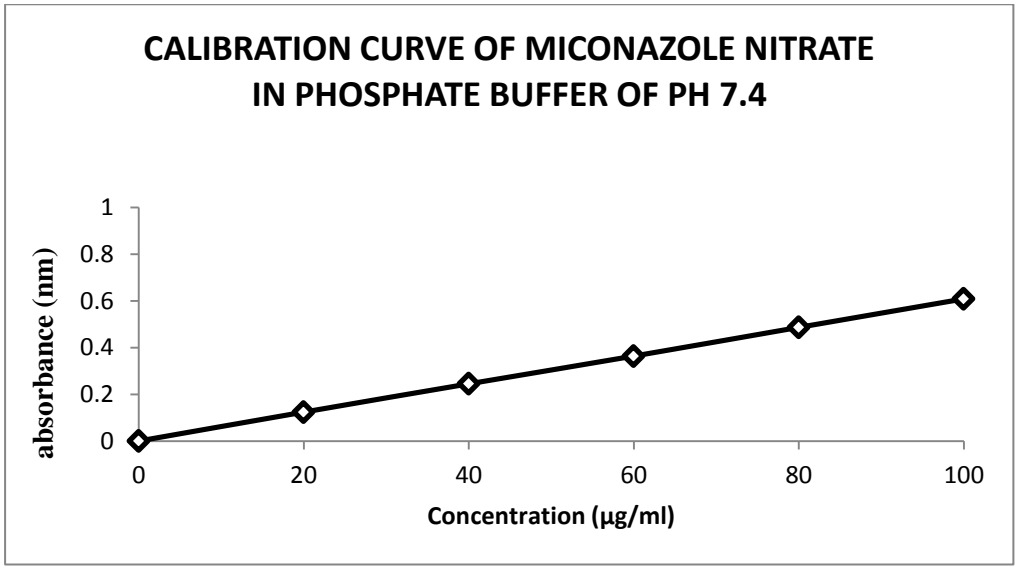

Figure 13: Calibration curve of Miconazole nitrate in phosphate buffer solution pH 7.4

6.2 PREFORMULATION STUDIES

6.2.1 Solubility studies of Miconazole nitrate

The result of solubility studies of pure Miconazole nitrate are given below:

Table No.14: Solubility studies of Miconazole nitrate

Solvents	Solubility (mg/ml)
Ethanol	0.78
Water	0.03
Acetone	0.36

From the data, solubility profile of Miconazole nitrate was insoluble in water, soluble in ethanol and acetone.

6.2.2 Melting point determination

The melting point was found to be 161^0C ± 0.577 and as per the IP 2007 melting point of Miconazole nitrate was within the range of 159-163 °C.

6.3 Drug excipient compatibility study

All the reference IR peaks of the pure drug Miconazole nitrate were also present in the spectra of mixture of drug- polymer and drug-permeation enhancer-excipients as mentioned in the above **Table No 10**.

So FTIR study showed that there is no interaction between drug and permeation enhancer. So the drug and permeation enhancer are compatible. The IR spectrums were given in the **Figures 14 to 20.**

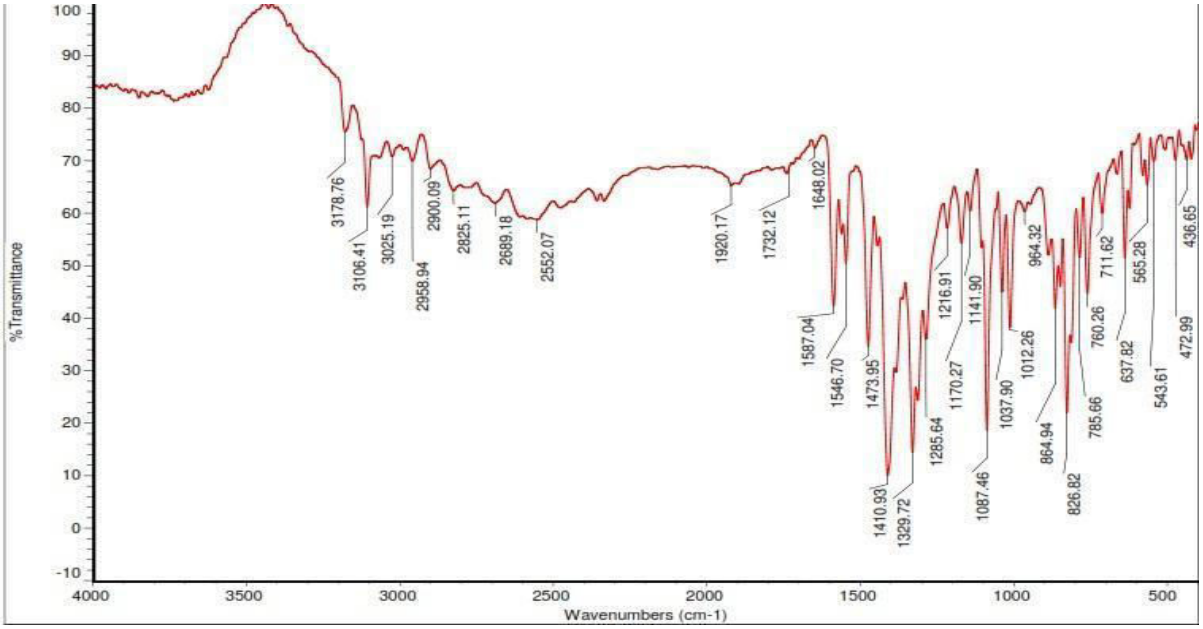

Figure 14: IR spectra of Miconazole nitrate

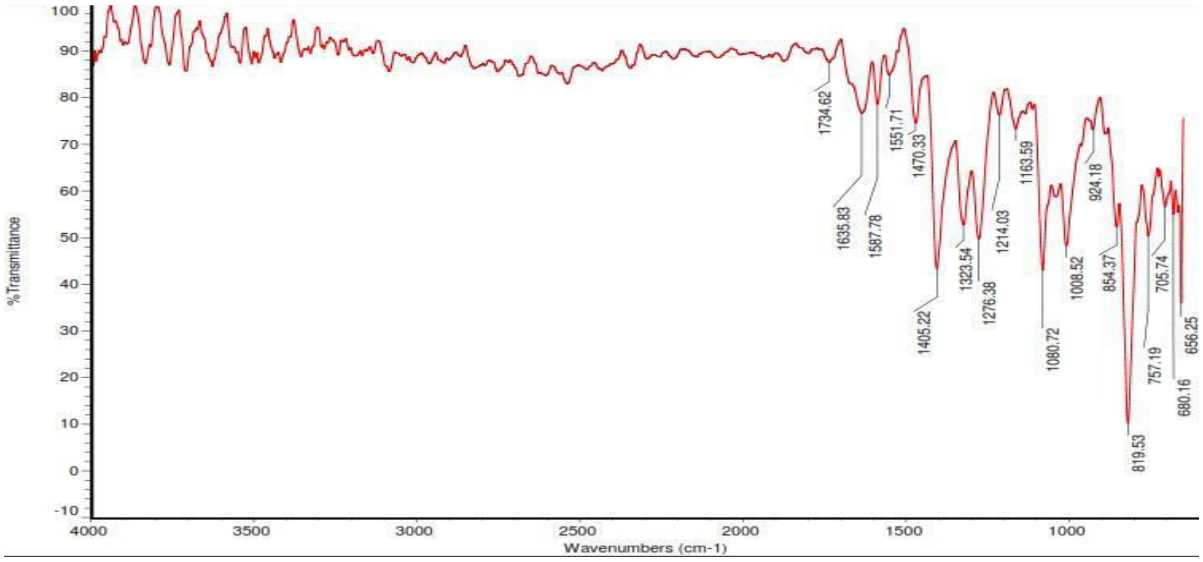

Figure 15: IR spectra of Nitrocellulose

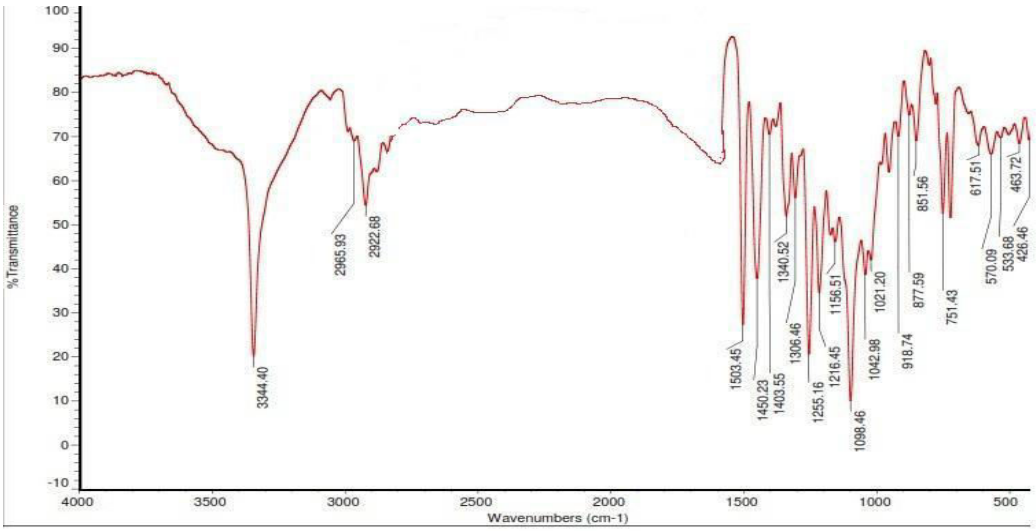

Figure 16:IR spectra of Ethyl cellulose

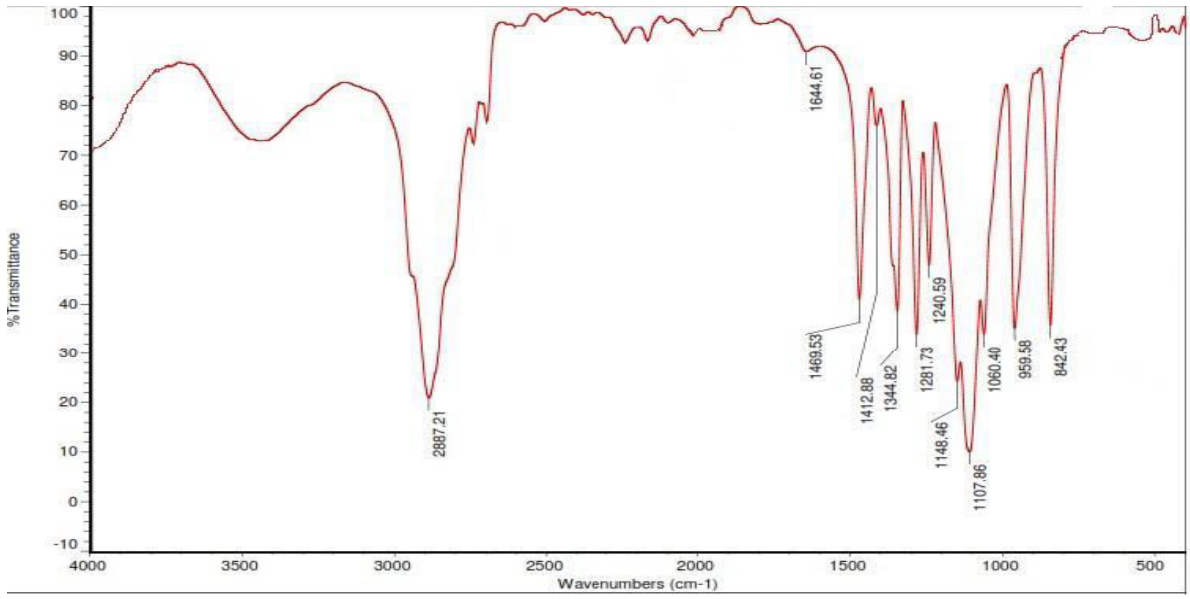

Figure 17: IR spectra of β- hydroxy Propyl Cellulose

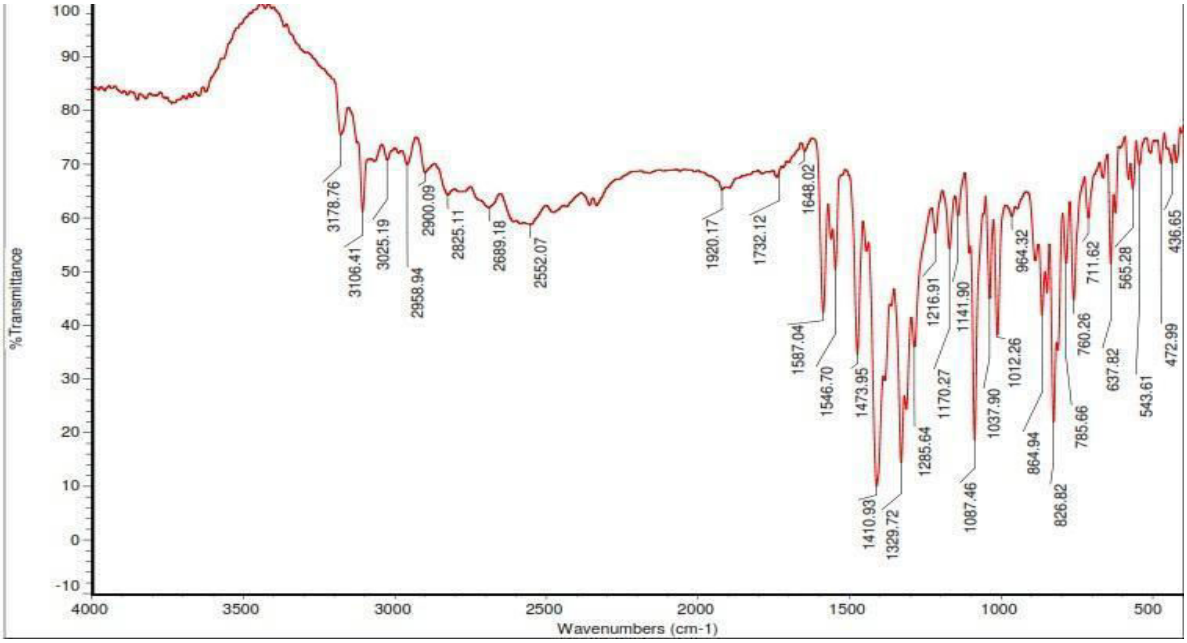

Figure 18: IR spectra of Miconazole nitrate and Nitrocellulose

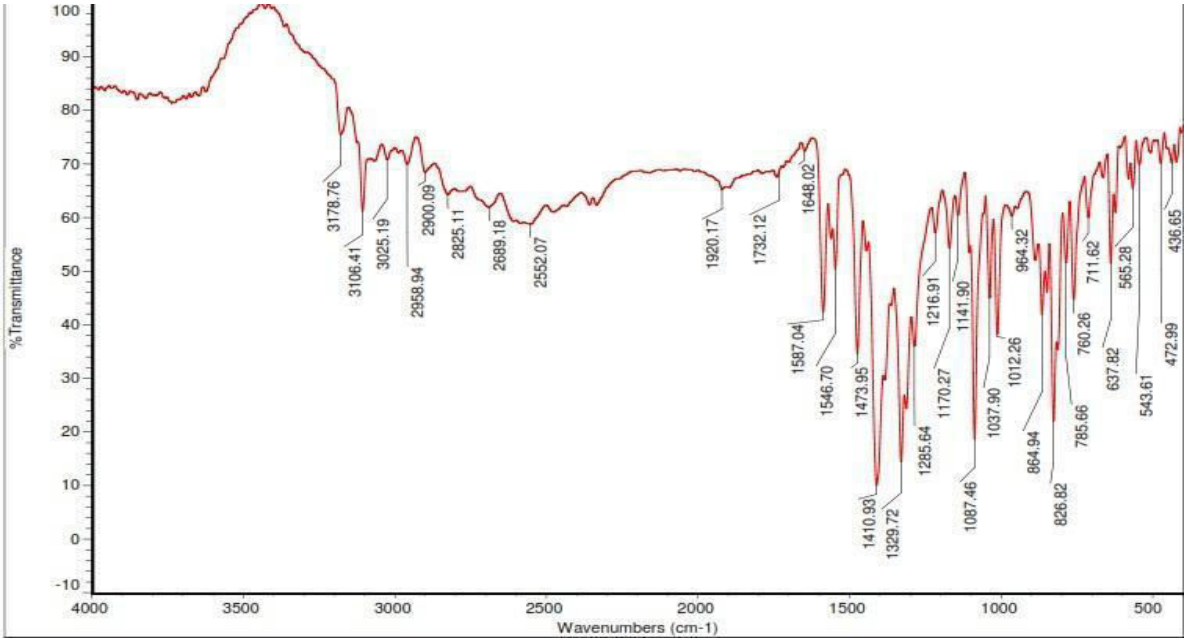

Figure 19: IR spectra of Miconazole nitrate and β- hydroxy Propyl Cellulose

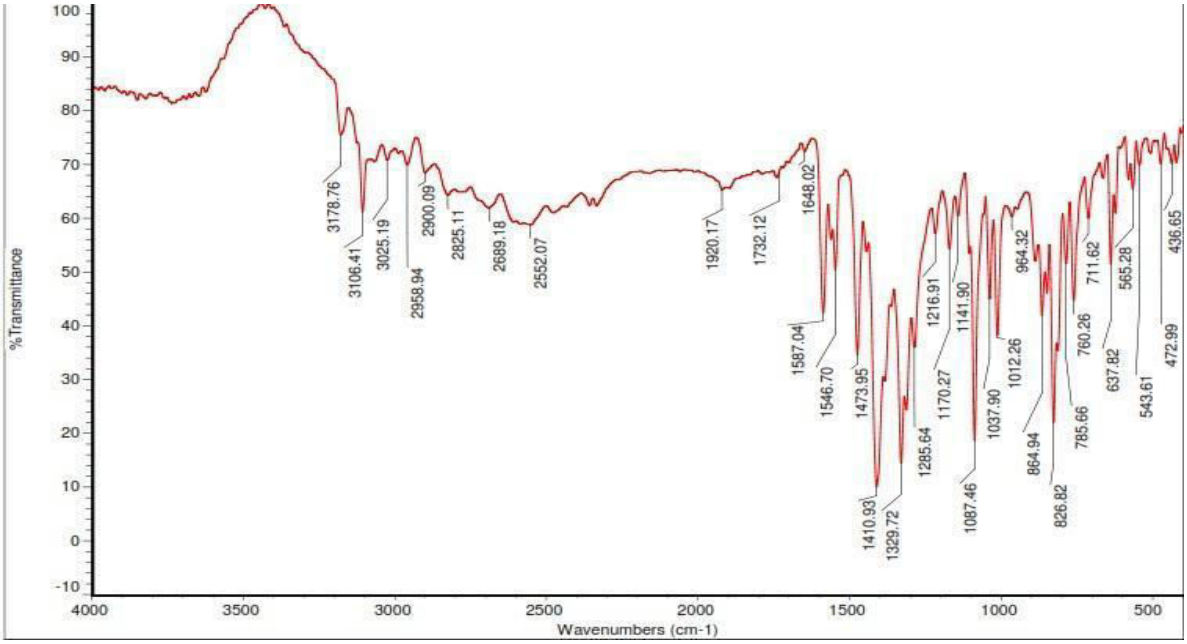

Figure 20: IR spectra of Miconazole nitrate Optimized Nail Lacquer Formulation (F11)

Table No.15: FTIR Compatibility study interpretation

FTIR spectra of pure Miconazole nitrate		**Miconazole nitrate Optimized Nail Lacquer Formulation (F11)**	
Wave number (cm^{-1})	**Functional group**	**Functional group**	**Wave number (cm^{-1})**
3281.15	Imidazole C–N stretch	Imidazole C–N stretch	3178.76
3254.78	Aromatic CH stretch	Aromatic CH stretch	3106.41
2972.93	Aliphatic CH_2 stretch	Aliphatic CH_2 stretch	2958.94
2885.64	Aliphatic CH stretch	Aliphatic CH stretch	2900.09
1448.73	$–CH_2–$ bending	$–CH_2–$ bending	1473.95
1416.04	C–H bending (aliphatic)	C–H bending (aliphatic)	1410.93
1329.03	C–N stretch	C–N stretch	1329.72
1083.82	C–C stretch	C–C stretch	1087.46
		C=C aromatic	1587.04
		C=C aromatic	1546.70
		C–H bending (aromatic)	711.62

After spectral comparison it was confirmed that no incompatibility reaction took place between drug and excipients, as all major characteristic IR peaks of Miconazole nitrate are present in the physical mixture with individual excipients and also in the final optimized formulation, F11. All the excipient peaks were found to be intact indicating good compatibility.

6.4 Formulation development of Nail Lacquer

The objective of the present study was to provide a formulation for inhibiting fungal growth on or underneath toe nails or finger nails so that the appearance of the nails are improved. Formulation includes a film former nitrocellulose, permeation enhancer such as 2-H- β-CD, keratolytic agent like salicylic acid and an antifungal agent (Miconazole nitrate) and ethanol as solvent. Formulation is prepared by simple mixing method.

Miconazole nitrate nail lacquers were evaluated on the basis of their film formation, smoothness of flow, drying time, gloss, nonvolatile content, viscosity and water resistance properties.

6.4.1 Optimization of nitrocellulose film former

Different concentrations of film forming polymers were used for film formation and then used for optimization of film. Different concentrations were tried between 2-8%. From the result, it was observed that by increasing the concentration of polymer up to 6%, thickness and strength of film was improved. When increasing concentration more than 6%, sticky films were formed. Thus, 6% concentration of polymer was used for further optimization of plasticizer. Plasticizer tried were Glycerin and Propylene glycol in 10% concentration each. Glycerin showed more sticky film which was unable to detach from surface. Propylene glycol showed good film forming property with good flexibility. Thus, 6% nitrocellulose and 10% propylene glycol, due to its excellent film forming nature was selected for further optimization studies.

A) Thickness (µm)

Uniform thickness indicates the uniformity of the formulations thereby suitability of the executed procedure. Thickness of all the films measured by using a micrometer screw gauge. Results showed that thickness of all formulations varied from 55 to 59 µm.

The observed values were enlisted in the **Table No.16**. Data for film thickness was matching within the desired range of thickness identified through review of literatures for films.

B) Folding endurance

Folding endurance indicates the flexibility of the polymer film. In order to evaluate the flexibility, the prepared films were subjected to folding endurance studies. The number of folds a film can sustain without break will dictate its folding endurance. The values obtained were above 125 in all of the developed films and are listed in **Table No.16** and it was in the range of 126-178 for all the developed films. Irrespective of polymer concentration used, all the films showed good folding endurance, revealed that the prepared films were having the capacity to withstand the mechanical pressure along with good flexibility. The folding endurance is an important evaluation, which ensures the flexibility of the developed films. Higher the folding endurance values better will be the flexibility of the films. 6% film (NF3) showed good folding endurance, thereby ensuring good flexibility.

C) Tensile strength (Kg/cm^2)

Tensile strength indicates the flexibility / elasticity of the film and its capacity to withstand a mechanical pressure. The tensile strength values depend on the percentage of the polymer solutions and polymer: plasticizer ratio. Tensile strength was measured by using an instrument designed and developed exclusively for the project. Since all the formulations have different proportions of polymers, from the data mentioned in **Table No.16** it was clear that 6 % concentration of nitrocellulose (NF3) showed higher tensile strength and the lowest average tensile

strength value was recorded for 8 % film.

Table No.16: Optimization of nitrocellulose film former

Nitrocellulose Concentration (%w/v)	1	2	3	4
Thickness (µm)	58±0.02	59±0.02	55±0.04	58±0.03
Folding endurance	155	126	178	177
Tensile strength (Kg/cm^2)	2.56±0.01	2.58±0.01	2.60±0.04	2.55±0.02

B) Water resistance

This is the measure of the resistance towards water permeability of the films. This was done by applying a continuous film on a surface and immersing it in water. The weight before and after immersion was noted and increase in weight was calculated. Higher the increase in weight lowers the water resistance. Here Nitrocellulose Film of 6% (NF3) has comparatively low weight and has the better water resistance. The datas were mentioned in **Table No.17**.

TableNo.17: Water (W) resistance of nail lacquers

Formulation code	**W_1(g)**	**$W_{2(g)}$**
NF1	6.85	6.92
NF2	6.83	6.95
NF3	6.88	6.90
NF4	6.92	7.14
NF5	6.81	6.90
NF6	6.84	6.91
NF7	6.89	6.94
NF8	6.90	7.04

Based on above studies it was decided that, NF3 formulation has the ideal characteristics required for a nail lacquer and hence 6% w/v of nitrocellulose and 10%w/v of Propylene glycol was concluded to be the optimum concentrations.

6.5. Evaluation of nail lacquer

All formulations showed desired film formation, smoothness of flow was good. Desired amount of nonvolatile matter (31 - 41%) was seen with complete evaporation of volatile matter leaving a thin film; the results were plotted in **Table No.18**. Drying time was found within 52 -127 sec. Except for F2, where it showed 127 sec, all formulations showed rapid drying rate. ie less than 60 seconds. The datas were mentioned in **Table No.19.**

A) **Nonvolatile content**

The non- volatile content of all formulations has been reported in the **Table No.18**, given below

Table No.18: Nonvolatile content of nail lacquers.

Formulation code	Non-volatile content (%)	Formulation code	Non-volatile content(%)
F0	33±0.38	F6	37±0.81
F1	33±0.38	F7	35±0.70
F2	41±0.81	F8	31±0.40
F3	39±0.40	F9	34±0.41
F4	37±0.81	F10	33±1.22
F5	35±0.71	F11	36±0.81

B) **Drying time**

Table No.19: Drying time of nail lacquers

Formulation Code	Drying time (sec)	Formulation code	Drying time (sec)
F0	50	F6	56
F1	52	F7	59
F2	127	F8	55
F3	52	F9	59
F4	58	F10	58
F5	59	F11	56

C) **Smoothness to flow and Gloss:**

Both these parameters was found to be satisfactory as can be observed from Fig. The nail lacquer poured onto the glass plate was found to spread and result in a uniform smooth film . The gloss of the applied lacquer was comparable with marketed cosmetic sample proving the cosmetic acceptance.

Figure 21: Smoothness to flow and Gloss of nail lacquer

D) **Viscosity**

The viscosity of the sample ranged from 100 to 220 centipoise and it was observed that between 140 to 160 centipoise the product was clear and glossy. More over this viscosity range provided good adherence and flow property. Viscosity outside this range produces clouding and decreases gloss which will not be cosmetically acceptable.

Table No. 20: Viscosity of nail lacquers

Formulation code	Viscosity	Formulation Code	Viscosity
F1	100	**F7**	200
F2	111	**F8**	140
F3	122	**F9**	142
F4	133	**F10**	146
F5	184	**F11**	152
F6	198		

E) **Adhesive strength**

The adhesive strength of the optimum batch was found to be comparable with marketed sample and hence can be expected to possess adequate adhesive strength on applied nail surface.

Table No. 21: Adhesive strength of nail lacquers

Formulation Code	Force of Adhesion (N)	Adhesive strength (N/m^2)
F11	0.5	12.5
MARKET SAMPLE	0.6	15

F) **Percentage drug content determination**

Percentage drug content for all the lacquers were found to be satisfactory and in between 86.25-99.01% which is reported in **Table No.22**. Highest % of drug content was found to be 99.01% (F11) and the lowest % of drug content was 86.25% (F3). Drug content more than 90% in the formulation shows the high amount of drug present in the formulation, ensuring that the methods of formulation and the ingredients selected are not affecting the stability of drug. High drug content also gives the assurance that, a good therapeutic outcome can be expected.

Table No.22: Percentage drug content

Formulation Code	Drug content (%)	Formulation code	Drug content (%)
F0	90.00	**F6**	89.35
F1	91.50	**F7**	90.10
F2	93.75	**F8**	98.0
F3	86.25	**F9**	98.22
F4	94.28	**F10**	97.55
F5	95.80	**F11**	99.01

G) **Diffusion studies across artificial membrane**

Diffusion studies of all the formulations were carried out using artificial membrane (cellophane membrane -0.8µm) for 48 hrs. The diffusion studies were conducted on all formulations as per given in **Table No.12.**

The first formulated batch F0 did not consist of any permeation enhancers and in vitro diffusion study revealed that only 27.10 % drug released till 48 hrs.Thus trials were planned to incorporate a permeation enhancer. Salicylic acid at concentrations of 5% (F1), 10% (F2), 15% (F3) and 20% (F4) was tried out. The diffusion studies revealed that only 64.18%, 65.10%, 68.34% and 69.10% respectively was released in 48 hours. It was clear that salicylic acid has improved the drug permeation due to its keratolytic activity. But it was also found that the drug permeation was not still complete and further increase in salicylic acid concentration is not expected to improve permeation. Hence it was decided to select 15% w/v of salicylic acid as the optimum concentration.

To further improve drug diffusion it was decided to include 2-H- β-CD in concentrations of 5% (F5), 7.5% (F6) and 10% (F7) into formulations. The drug release and diffusion across membrane was found to improve in presence of 2-HP- β-CD. At concentration of 5%, 82.40% diffusion in 28^{th} hour was observed. In case of F6, 89.0% diffusion was observed at 28^{th} hour. It was also observed that as concentration of 2-HP- β-CD increased drug diffusion also improved drastically as clear from almost complete drug diffusion of 98.40% release in 20^{th} hour with 7.5% concentration.

Though, inclusion of 2-H-β-CD has improved drug diffusion to 98.40%, it was observed that the release was found to be complete within 20 hours. Therefore to sustain the drug release over an extended period it was decided to include a rate controlling polymer ethyl cellulose at concentrations of 0.25% (F8), 0.5% (F9) and 0.75% (F10) and 1.0% (F11) into formulations. The result showed an extended and complete drug release of 96.80% at 28^{th} hr. in F8 and 93.0 % till 36^{th} hour in F9. In F10, a drug diffusion of 97.20% was observed at 40^{th} hr. And finally when the concentration of ethyl cellulose was increased to 1% in F11, a drug diffusion of 98.12 percent which sustained over a period of 48 hours was achieved.

The formulation F11 was selected as the optimized nail lacquer formulation based on drug diffusion studies.

Table No.23: Comparative study and optimization of salicylic acid concentration

Time(hr)	PERCENTAGE DRUG RELEASE (µg/ml)			
	F1	**F2**	**F3**	**F4**
0	0	0	0	0
2	9.82	11.22	13.35	15.25
4	10.20	12.05	14.98	16.88
6	13.28	14.35	16.35	17.22
8	16.42	17.88	18.85	20.13
10	26.58	28.95	32.05	30.35
12	32.45	36.33	40.20	36.15
16	43.10	42.30	48.38	42.95
20	48.22	49.98	51.80	50.10
24	49.65	50.80	52.61	54.32
28	52.55	54.89	56.80	58.38
32	56.25	58.75	59.33	60.21
36	58.95	59.98	61.28	63.45
40	60.18	62.15	63.92	66.21
44	62.52	63.25	65.99	68.84
48	64.18	65.10	68.34	69.10

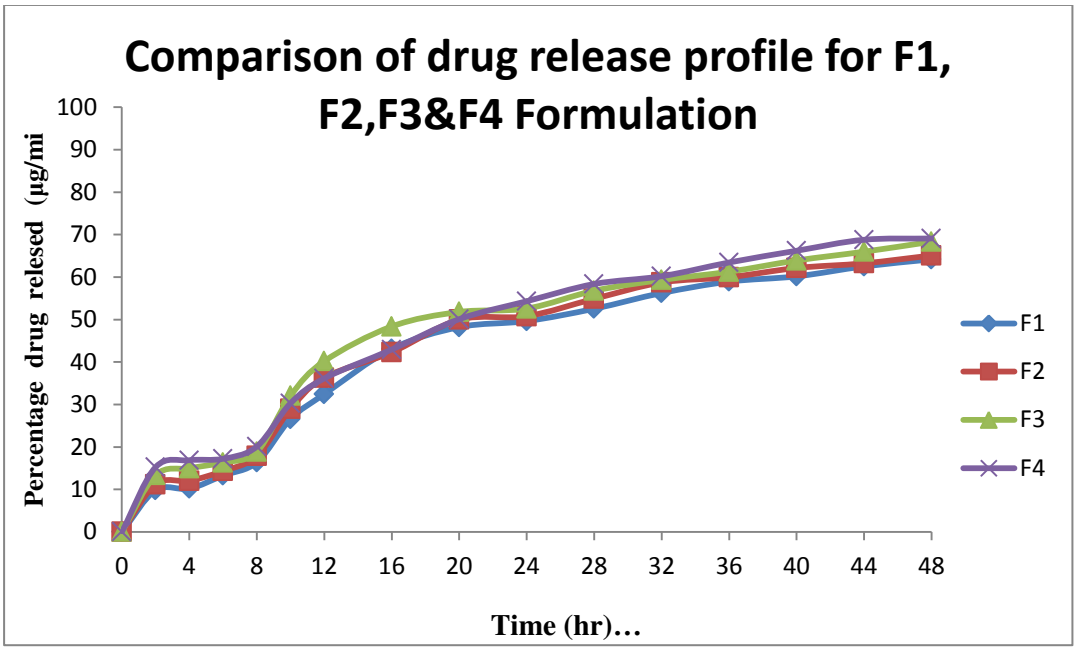

Figure 22: Comparative Dissolution profile of F1 v/s F2 v/s F3 v/s F4

Table No.24: Comparative study and optimization of 2-HP- β-CD concentration

Time(hr)	Percentage drug release		
	F5	F6	F7
0	0	0	0
2	26.25	32.12	39.31
4	32.23	43.55	49.85
6	38.51	52.82	59.65
8	46.52	61.65	67.72
10	48.22	69.35	76.45
12	56.28	76.25	85.05
16	65.15	80.02	92.15
20	76.45	83.35	98.40
24	79.92	88.95	96.25
28	82.40	89.00	94.23
32	80.25	86.32	93.15
36	79.45	84.15	91.82
40	77.31	82.15	90.08
44	76.65	80.85	89.16
48	74.72	78.25	88.95

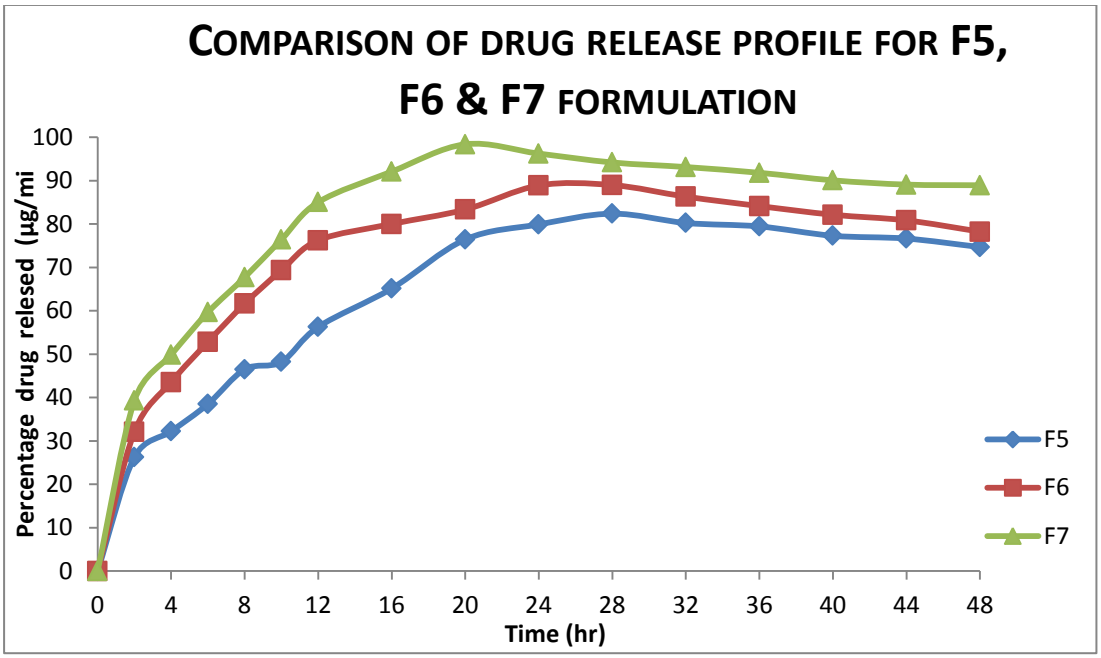

Figure 23: Comparative Dissolution profile of F5 v/s F6 v/s F7

Table No.25: Comparative study and optimization of Ethyl cellulose concentration

Time(hr)	PERCENTAGE DRUG RELEASE (µg/ml)			
	F8	F9	F10	F11
0	0	0	0	0
2	29.65	26.52	19.45	12.82
4	34.12	31.98	30.45	27.12
6	45.56	40.43	36.91	28.31
8	51.16	44.90	48.84	32.72
10	62.34	53.22	50.74	46.25
12	69.74	60.13	56.79	50.21
16	75.93	68.66	60.24	58.65
20	88.45	72.32	65.71	60.21
24	93.23	83.45	72.67	68.11
28	96.81	89.76	80.51	70.22
32	95.00	95.84	85.72	78.85
36	94.57	93.78	90.62	84.15
40	93.14	90.72	97.56	88.85
44	90.76	89.87	94.22	90.25
48	89.01	88.73	91.31	98.12

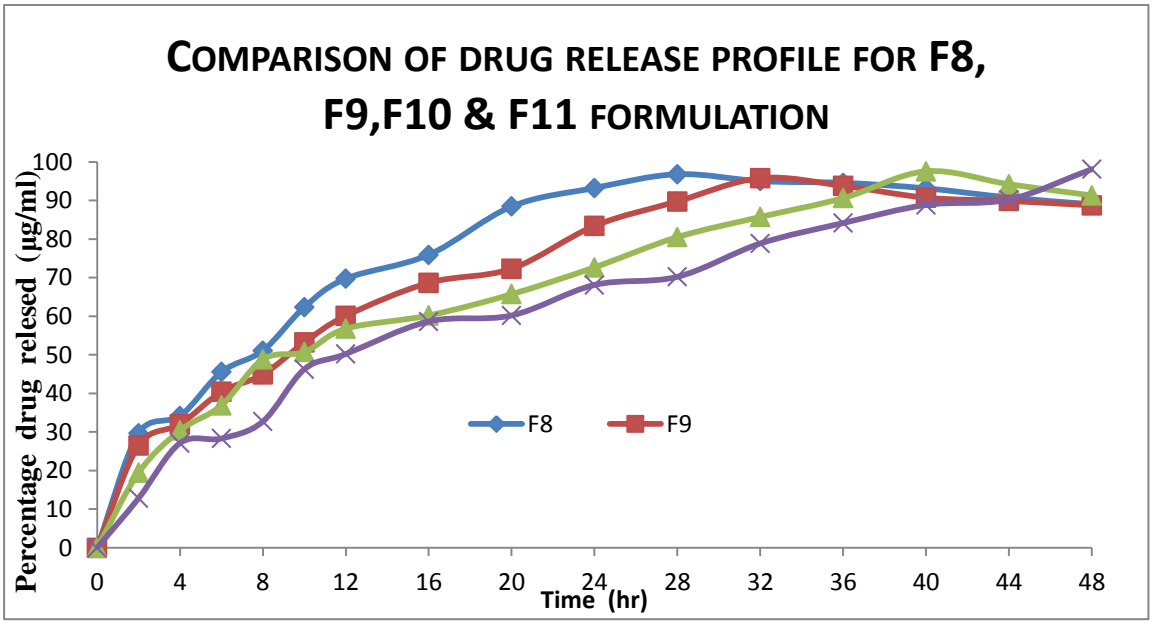

Figure 24: Comparative Dissolution profile of F8 v/s F9 v/s F10 v/s F11

H)***In vitro*** **ungual permeation studies**

To simulate and mimic diffusion study with that of *in vivo* conditions, i.e. across nail plate, a diffusion study across hooves obtained from freshly slaughtered cattle was done. There was no significant difference in diffusion and drug release data obtained across artificial and hoof's membrane. This study gives the assurance that a good *in vitro in vivo* correlation can be expected.

Table No.26: Comparison of drug diffusion across artificial membrane and hoof's membrane

Time	PERCENTAGE DRUG RELEASE (µg/ml)	
	% drug diffused through artificial membrane	**% drug diffused through hoof's membrane**
0	0	0
2	12.82	14.50
4	27.12	20.90
6	28.31	26.45
8	32.72	36.75
10	46.25	47.90
12	50.21	56.72
16	58.65	60.45
20	60.20	65.80
24	68.11	72.55
28	70.22	80.60
32	78.85	85.05
36	84.15	89.25
40	88.85	92.30
44	90.25	95.01
48	98.12	97.45

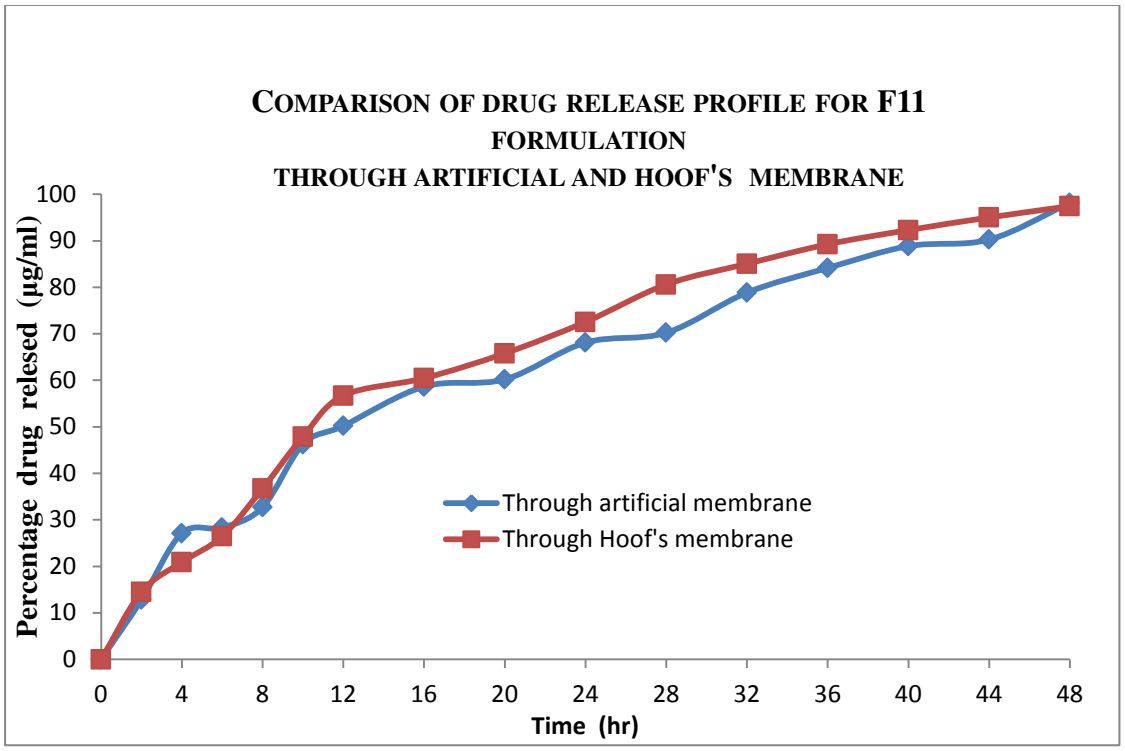

Figure 25: Comparison of drug diffusion across artificial membrane and hoof's membrane

I) **Anti-microbial study**

The zone of inhibition for the various formulations was determined, and it was found to range from 17-22mm, which is comparable with that of standard with 21mm. This indicates that all the formulations were sensitive to the microorganism *Candida albicans* .Results are reported in **Table No.26** and **Figure:26.**

Figure 26: Zone of inhibition of Formulated Nail Lacquers

Table No.27: Zone of inhibition of Miconazole nitrate Nail lacquers

Formulation Code	Zone Of Inhibition (mm)	Formulation code	Zone Of Inhibition (mm)
F1	22	**F7**	19
F2	18	**F8**	24
F3	21	**F9**	17
F4	22	**F10**	23
F5	17	**F11**	22
F6	16	**Standard**	21

J) **Stability studies**

Stability studies were used to determine the shelf life and storage condition of a product. In this investigation F11 were subjected to accelerated stability studies for a period of 1 month. Accelerated stability studies were performed in accordance with ICH guidelines with necessary modifications.

The studies were carried out to verify the changes in physical characteristics such as Non -volatile content, Drying time, % drug content, drug diffusion at three different conditions of higher temperature (40 ± 2^0C) for 1 month. The results are reported in **Table No.28,29.**

Table No.28: Stability studies data of F11

Parameter	Initial	After
Non-volatile content	36±0.81	35±0.35
Drying time(sec)	56	58
Drug content	99.01	98.50

Table No.29: Invitro Diffusion profile of F11 upon stability studies

Time	PERCENTAGE DRUG RELEASE (µg/ml)	
	Before stability	After stability
0	0	0
2	12.82	10.60
4	27.12	24.90
6	28.31	26.45
8	32.72	30.25
10	46.25	39.95
12	50.21	45.75
16	58.65	52.55
20	60.20	58.81
24	68.11	62.50
28	70.22	72.05
32	78.85	76.80
36	84.15	81.25
40	88.85	90.53
44	90.25	92.20
48	98.12	97.75

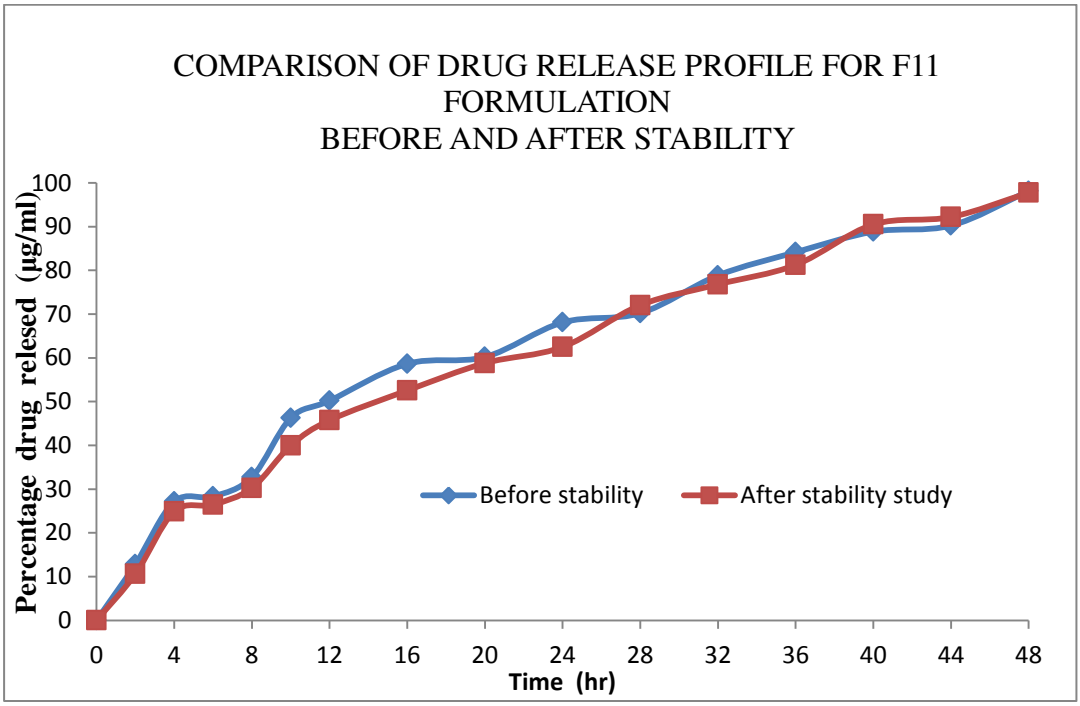

Figure 27: Invitro Diffusion profile of F11 upon stability studies

The evaluation of formulations after stability charging showed there was no significant change with respect Non -volatile content, Drying time % drug content and drug diffusion with respect to results obtained before stability charging. Thus it was concluded that the formulations were found to possess stability compliance requirements as per ICH guidelines.

7. SUMMARY

The objective of the present work was to formulate a medicated antifungal nail lacquer containing Miconazole nitrate for the treatment of Onychomycosis. Conventional treatments for onychomycosis topically can clinically not efficient, as formulations must permeate the nail barrier in order to deliver therapeutic levels of active agents to the target site. In the present study, nail lacquer containing a keratolytic agent salicylic acid and a permeation enhancer (**2-Hydroxypropyl)-β-cyclodextrin** in different concentration are tried out and comparison of extent of drug permeation has been done among the same.

Initially, research work started with a wide and through literature survey followed by

- Analytical development studies, where identification and determination of λmax and preparation of calibration curve was done. Standard curve was prepared for determination of Miconazole nitrate in phosphate buffer pH 7.4 at 223nm straight line equations for calculations were derived.
- Preformulation studies of drug like determination of solubility, melting point and excipient compatibility studies by FT-IR.
- During Formulation development studies, a total eleven formulations of nail lacquer using permeation enhancers (**2-Hydroxypropyl)-β-cyclodextrin** and keratolytic such as Salicylic acid, were prepared using simple mixing technique. Various trials with ethyl cellulose as a rate controlling polymer to sustain drug release over 48 hours were also tried out

Various studies on physicochemical parameters like film formation, optimization of salicylic acid concentration, non -volatile content, drying rate, gloss, viscosity, smoothness of flow, anti- microbial studies and stability studies were evaluated. All the above parameters were checked to find the compliance.

In vitro permeation studies were also performed. Studies were carried out using artificial membrane and bovine hooves membrane for 48 hrs. to select the best formulation.

The best optimized formulation was further subjected for accelerated stability studies as per ICH conditions of 40 ± 2^0C for 1 month .The studies were carried out to verify the changes in physical characteristics such as Non -volatile content, Drying time, % drug content, drug diffusion under influence of temperature while on storage.

8. CONCLUSION

- The purpose of the present investigation was to formulate and evaluate the Miconazole nitrate nail lacquer as an ungual drug delivery system for the treatment of onychomycosis.
- Miconazole nitrate was chosen as a model drug, the formulations were prepared with permeation enhancers (**2-Hydroxypropyl**)-**β-cyclodextrin** and keratolytic agent, Salicylic acid. Then, these lacquers were compared for drying time, nonvolatile content drug content, drug diffusion and anti -microbial studies.
- From the FTIR studies, it was concluded that the drug and the excipients used in the formulations were compatible with each other. .
- All formulations showed good film formation, drying time, smooth flow, and required volatile content.
- Microbial study results proved that the formulations are sensitive to the microorganism *Candida albicans.*
- The stability tests showed that the formulations were stable at 40^0c for 1 month.
- From Invitro ungual permeation study a good invitro in vivo correlation can be expected.
- The results obtained from the *in vitro* studies indicate that formulation F11 showed a complete drug release which sustained over 48 hours. The F11 formulation had salicylic acid at concentration of 15% w/v as keratolytic agent and 10% w/v of (**2-Hydroxypropyl**)-**β-cyclodextrin** as permeation enhancer. This indicates that the combination of permeation enhancer and keratolytic agent resulted in an improved permeation rate and also a complete and sustained drug release.
- The percentage non-volatile content of F11 formulation was found to be 36 ± 0.81.The desired amount of non-volatile matter was seen with complete evaporation of volatile matter.
- F11 formulation showed rapid drying rate.
- The viscosity of F11 formulation was observed as 152.so this formulation was clear and glossy.
- The adhesive strength of F11 formulation compared with marketed sample and it possess adequate adhesive strength on applied nail surface.

- 99.01% of drug content was found in F11. So a good therapeutic outcome can be expected.
- From Diffusion studies across artificial membrane, the inclusion of **2-Hydroxypropyl)-β-cyclodextrin to F11** has improved drug diffusion to 98.40%.
- The formulation F11 was selected as the optimized nail lacquer formulation based on drug diffusion studies.
- Stability study data showed that there was no much change in the values after stability test. It was concluded that the formulations were found to possess stability compliance requirements as per ICH guidelines.

From the above studies, it can be concluded that medicated nail lacquers proved to be a better tool as a drug delivery system for the ungual drug delivery of an antifungal in the treatment of onychomycosis. Apart from treating the nail infections, the medicated nail lacquers can be also used for beautification of nails with ease of application. This improves patient compliance and acceptability.

Future Prospective

Though in this study it has been proved that medicated nail lacquers can act as a useful tool for the ungual drug delivery of an antifungal in the treatment of onychomycosis, further clinical and pharmacokinetic studies are required to explore the potential of this system for use in humans.

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